

Reprinted from
Acta Societatis Medicorum Fennicæ "Duodecim", Ser. A, Tom. XVI, Fasc. 2

ON THE CONTINUOUS
OLIGODYNAMIC EFFECT OF
ELEMENTS ON BACTERIA

ACADEMICAL TREATISE

BY

Y. SEUDERLING

LIC. MED.

HELSINKI 1933

From the State Serum Laboratory, Helsinki

ON THE CONTINUOUS
OLIGODYNAMIC EFFECT OF
ELEMENTS ON BACTERIA

BY

Y. SEUDERLING

LIC. MED.

*With the sanction
of the Medical Faculty of the University of Helsinki
submitted to official disputation at the hist.-philolo-
gical auditory, on May 29th 1933 at 12 o'clock noon.*

HELSINKI 1933

PRINTED BY
SUOMALAISEN KIRJALLISUUDEN SEURAN KIRJAPAINON OSAKEYHTIÖ
HELSINKI 1933

Contents.

	Page
Chapter 1. Introduction	7
» 2. The effect of elements on live cells, earliest tests	11
» 3. The effect of metals and chemical compounds on bacteria ..	14
» 4. The effect of metals and chemical compounds in regard to fungi and protozoa	26
» 5. The physical power of elements and their radiation in regard to oligodynamics	33
The effect of colloidal elements on bacteria	
	39
The effect of radioactive substances on bacteria	41
» 6. The effect of electrolytes in oligodynamics	43
» 7. The effect of different elements in connection with their place in the periodic system	47
» 8. Activation of glass vessels in oligodynamic experiments	50
» 9. Discoloration of bacteria under the influence of physicochemical agents	52
» 10. The author's experiments	54
» 11. Conclusions	110
References	111



Digitized by the Internet Archive
in 2026

https://archive.org/details/IA40153320_0221

Preface.

The present work has been accomplished at the State Serum Laboratory under the supervision of J. A. MURTO, M. D., Physician in Chief at the laboratory. It is my pleasant duty to thank him for the interest taken in my work and for the advice and support given me in the course of my investigations. My further thanks are due to G. KOMPPA, Professor of Chemistry at the Technical High-School, for allowing me to use his laboratory for the necessary analyses.

The author.

CHAPTER I.

Introduction.

There are problems in biology which seem simple enough at first sight; a closer investigation, however, often reveals considerable difficulties, especially when one has to explain the causes of observed phenomena. Such a problem is met with when studying the question of the so-called oligodynamic influence of chemical elements and compounds on various kinds of live cells. The cause of oligodynamic action has been understood in different ways, this being chiefly due to the varying conditions in which the experiments were performed. Investigations of the various elements' oligodynamic action has been pursued along two main lines viz.: 1) Substances were allowed to affect bacteria in ordinary petri dishes in agar or gelatine cultures; this method makes it possible to watch the different phases of the culture and to photograph results. 2) Substances were allowed to act in water or solutions of salts at the same time as the micro-organisms were contained therein. In certain cases the water was first »activated», especially when carrying out the experiments with pure metals. The metal was allowed to lie in water before the cells were added to it. Some scientists have used metallic salts, others elements. Numerous experiments have been made with colloidal elements and even radioactive substances were used. ELFVING allowed metals to act at some distance from the cells. In 1931 GOTTSCHALK proposed a standard method for oligodynamic tests. A German 5-mark coin, thoroughly cleansed in alcohol and ether, is put into redistilled water or such sterilized through Berkefeld filter, whereafter the germicidal properties of the fluid were tested. Owing to varying conditions prevailing during the experiments, the results of these tests, performed according to different methods, can not be compared.

The physicochemical characteristics of different media are already at variance; dissolved particles can easily move in a solution

and reactions take place between active substances and micro-organisms in a molecularly dissolvable medium. If the tests are made in agar one has to do with a colloid which is of a considerably complicated composition, thus offering less opportunity for reaction between the different substances. The agar can even be considered as a protective colloid, diminishing the action of substances. According to KRÖNIG's and PAUL's investigations the disinfecting property of a salt stands in proportion to the number of ions formed during the process of dissolution.

Some errors can not be entirely avoided. In cases where glass vessels are used for solutions and metals or metallic salts, the chemical nature of the glass alone can be of consequence; a chemical reaction may take place between the glass and the substance under test, or else a potential difference may be at hand. The salts contained in the agar can also influence the diffusion. According to BECHOLD and ZIEGLER the permeability of a colloidal medium may be altered by different substances. Those that raise the boiling point of a colloid resist diffusion, and those that lower it are diffusion promotive. Agar and gelatine diminish in a marked degree the diffusion of both electrolytes and non-electrolytes. NaCl and NaJ, however, do not influence noticeably a diffusion in 1—2—4 per cent agar, NaCl is even inclined to accelerate the diffusion. The presence of either electrolytes or non-electrolytes, however, influences the diffusion of a third substance in agar. Moreover there is still the effect, at least in solutions, of the difference between the electric charge of the micro-organism and that of the surrounding medium or substance dissolved therein. Bacteria, when in suspension, are negatively charged (NORTHROP and DE KRUIF, KARCZAG), whereas, e.g., a metal ion is positively charged. When using chemical elements one should always consider their solution-pressure (KRUYT) which is important during any work with solutions; this should also be observed in a certain degree when performing tests in agar, owing to the fact that it contains water.

PAUL SAXL is a scientist whose opinion on oligodynamics of elements has raised much opposition which in its turn has excited greater interest in the study of this subject. In this connection it should only be mentioned that, according to his ideas, the oligodynamic action in respect of elements is primarily dependent upon a physical power acting on the surface of the metal and originating from the air.

Tests made *in vitro* cannot be compared with such made *in vivo*, because in the latter case numerous other phenomena arise which it is difficult to determine exactly. At this point reference should be made to WALBUM's extensive therapeutical experiments with metallic salts. LOUROS and SCHEYER, 1927, maintain that the use of metals in therapeutics is nothing new and that during the last few years considerable attention has been paid to oligodynamics, the nature of which is still unknown. ROOSEN, 1930, is of the opinion that the therapeutic effect of metallic compounds in certain diseases depends upon the slow resorption of metal with oligodynamic action in the form of an internal antiseptic. According to LIESEGANG oligodynamic action sets in during an intermediary dissolution of practically indissoluble metals and metallic salts. This plays an important rôle in therapeutics with colloidal silver and explains its damped, prolonged action in contrast to the effect of true solutions. According to V. KORANYI and RICHTER the oligodynamic action of metals depends on their uniting with the albumen of the micro-organism, causing a chronic metal poisoning. In the opinion of PAULI and FLECKER the salts of heavy metals assimilate with albumen to form metallic albuminates, probably through the splitting away of metallic hydroxides. BUCHSTAB, 1928, explains oligodynamic action as a series of physicochemical processes in live i.e. not as a chemical action of inorganic substances. EICHBAUM, 1932, gives the definition that oligodynamic action is the effect of minute quantities of positively charged metal ions. HODER, 1932, has found oligodynamic action in water distilled in a copper apparatus and none in such from a glass one. Oligodynamic action is more likely to impede the growth of bacteria than to be bactericidal in itself: this depends on minute quantities of dissolved metal. According to KONRICH, 1931, the germicidal effect of pure metals in water during the sterilization process of drinking water has already been used in practice with silver and been given the description »catadynic process». PILOD and CODVELLE, 1932, however, are of the opinion that copper is an insufficient disinfectant for drinking water owing to its comparatively slow action. It can still be used to sterilize rain- and spring-water.

I have assumed in my tests that the oligodynamic action of various elements is harmful to micro-organisms. MURTO has shown that many elements possess certain characteristics which stimulate the growth of bacteria. Those elements are found in BOHR's table (KRAMERS and HOLST) above Mg, which element both impedes and

stimulates the growth of a bacteria. The majority of scientists who have made their tests in agar or gelatine cultures are of the opinion that such bacteria, as are nearest the element, within the region, which is seemingly free from bacteria growth, are dead. Only a few, like BEHRING and MESSERSCHMIDT, have investigated whether this is really the case, but even they have not made any systematic researches in this trend. MURTO has found that the harmful oligodynamic effect is first of all injurious; bacteria do not divide within the seemingly growth-free region, but their lifetime differs and when transferred onto fresh culture media they can propagate again. *B. pyocyaneum*, when kept in a vessel with screw lid, was alive in agar with Sb during 6 months. HODER maintains that even in water solutions the deleterious action is the principal one. The tests discussed were made to ascertain the life length of bacteria with different elements. It was also interesting to study the way the elements acted and to observe such changes in certain cultures, as were noticeable to the bare eye.

The method is fully described in the experimental section. The order of questions was the following:

How long do different bacteria live with different elements in agar? Can the elements, or some of them, be traced in the culture media? Do biological changes take place in the function of bacteria in general, or some of these in particular?

CHAPTER 2.

The effect of elements on live cells, earliest tests.

One of the earliest publications dealing with the influence of metals on live cells is that of MILLER, 1889. Whilst experimenting with pepton-water-gelatine he found that some kinds of gold used for filling teeth prevented the growth of bacteria. An Au-Sn alloy did not produce the same effect as Au alone; Sn and Pt were passive in this respect, as were also metals which had been once before heated to a glow. He assumed at first that this effect of metals was due to small quantities of metal dissolved by the chlorides of the culture media. Later on he ascribed this effect as due to oxygen condensed on the surface of the metal becoming free and killing the bacteria. Nevertheless he found a contradiction in the fact, that finely spread metal did not increase the effect, also that metals, heated to a glow, even after 8 days, did not recover their antibacterial properties. He presumed the reason for this was some substance which must have been added to the gold in its manufacturing stages. This would also explain the different effect of various preparations. MILLER was never able to give a satisfactory explanation to these observations.

ELFVING, 1890, allowed metals to act at some distance on *Phycomyces nitens* which, owing to its rapid growth, is very suitable for such purposes. The metal pieces above the cultures acted as attractors, the *Phycomyces* inclined towards the metal and even at a distance of 2 to 3 cm. said attraction was still noticeable. Fe yielded the most pronounced effect, whereas Zn and Al proved uncertain. Ag, Au, Pt, Bi, Sb, Cd, Co, Ni, Sn, Pb and Cu exercised no effect at all. ELFVING was of the opinion that it was a question of vibrations emanating from metal molecules and spreading over the neighbourhood; only future investigation would bring a definite answer to this question. In the course of experiments taken up again in 1894 he found that highly polished steel and Pt developed attracting qualities when being exposed to light. The ultraviolet part of the spectrum is not necessary to bring

it about. The effect of metals comes from vibrations emanating from their molecules, a kind of phosphorescence, unperceivable to the naked eye. In 1916 he continued his experiments and found that Fe attracted the *Phycomyces*, acting at the same time as a check against increased growth lengthwise. Zn and Al also showed a definite effect. Metals which proved inactive during his first tests had no effect in this instance either. He came to the conclusion that air oxygen condensed itself on the surface of metals into ozone.

In 1893 appeared a posthumous paper by CARL v. NÄGELI, revised by SCHWENDENER. It seems that as early as in the eighties the former had investigated the action of small quantities of metallic salts and their effect on different kinds of fresh water alga *Spirogyra*. He found that they die at once if there is a certain concentration of the salt which has a chemically poisonous effect. The chlorophyll strings alter their shape without leaving the walls. A certain extremely weak concentration produces other changes in the cells before their death, chiefly characterised by loosening of the chlorophyll strings from the plasma tube, which does not alter its position. v. NÄGELI described these alterations as being oligodynamic; they are first of all morphologic. He divided the action of poisons into three groups: physical, chemicopoisonous and oligodynamic. Many substances, such as sugar and alcohol in larger quantities, cause physical changes which appear in the form of plasmolysis. In accordance with their concentration different substances cause either chemicopoisonous or oligodynamic changes, as is the case with different metals and metallic alloys. He performed his experiment with a solution given by Löw: 1 AgNO_3 , 1 NH_3 and 3,6 K_2O to 100000 parts of water. When the solvent was increased to a proportion of 1: 10^{24} the algae died, often within 3 to 4 minutes. By use of a sublimate solution oligodynamic changes began to take place at 1: 10^6 and by going up to 1: 10^{12} and 10^{36} oligodynamic changes in the cells became sometimes apparent within 3 to 6 minutes, but often also after 1 to 2 hours. At 1: 10^{42} oligodynamic changes in cells could still be observed. In some series of experiments with 1: 10^{18} and 1: 10^{30} solutions no oligodynamic changes could be detected. v. NÄGELI was not satisfied with these paradoxical results. After some unsuccessful experiments he found that the distilled water which is used as solvent has oligodynamic characteristics of its own. An examination of what was left of 10 ltrs proved the occurrence Pb, Zn, Cu and Fe. Water distilled from glass to glass was inactive. When such water was used to dissolve an oligo-

dynamic solution, a limit to the oligodynamic action was reached, which in the case of copper nitrate was found to be in a solution of 1 : 10⁸. Certain pure metals give oligodynamic properties to the water in which they lie. Such metals are Cu, Ag, Pb, Sn, Fe and Hg. Cu is soluble in water in a proportion of 1 : 77 millions; the metal is probably soluble as Cu (OH)₂. Other metals dissolve in the same way. 1 part Cu to 10⁹ parts water acts oligodynamically. Au and Pt exercise no effect and may be considered as insoluble. v. NÄGELI comes to the conclusion that the oligodynamic action of water is in any case a result of substances being dissolved therein. Oligodynamic changes are produced by the same substances that have a chemically poisonous effect; in the latter case, however, a larger quantity is required. Glass vessels filled with oligodynamically acting water retain this action even after having been cleansed and refilled with fresh water. Said effect decreases every time the process is repeated. This is due to the absorption of metal particles by the glass, which are being subsequently returned to fresh supplies of water. The oligodynamic action is prevented or suppressed by different kinds of colloidal substances. The same thing happens if the quantity of algae is excessive; metal particles in water become absorbed by a surface, which is enormously enlarged by the different colloids. These observations, in respect of »activated» vessels and the preventing action of colloids, have been later confirmed by a great many scientists.

CRAMER has added a few remarks to v. NÄGELI's treatise. He points out the following incredible solutions involved: should 1 mg. sublimate be dissolved in water in a proportion of 1 : 10⁴² a cube of water is necessary with a side-length of 13 millions geographic miles. Thus all the water on earth would not suffice to produce the desired solution.

In 1897 v. NÄGELI's experiments were continued by ISRAEL and KLINGMANN, who confirmed them; they used three kinds of *Spirogyra*.

Similar experiments made with *B. typhi* showed that after 1/2 to 1 hour the growth of bacteria was prevented and no new colonies formed after 2—24 hours. This was controlled on agar. The growth of *B. coli* and *Vibrio cholerae* was impeded after 1/2 to 2 hours and when 3 to 24 hours had elapsed the bacteria were dead.

CHAPTER 3.

The effect of metals and chemical compounds on bacteria.

MILLER's experiments attracted attention and lead to similar tests in order to check his observations. BEHRING, 1890, refers to them and states that if some substance is to be used for bactericidal purposes, it is necessary that it should act directly on the bacteria. Furthermore, to achieve this effect, it is necessary that the substance should be soluble in the medium containing the bacteria; only then can the molecules act on the micro-organism. The expression »corpora agunt nisi soluta» is confirmed and it is therefore of quite considerable interest that experiments have been made which actually seem to contradict it. He found that so-called cylinder gold in a gelatine culture of *B. anthracis* produces a concentric region, 1.5 cm wide, in which no bacteria growth is noticeable. With *Corynebact. diphtheriae* the region proved to be 3 to 5 cm wide, with *Vibrio cholerae* 0.4 cm. *B. mallei* and *B. typhi* were not influenced. Different bacteria react in different ways. Mintage, Au, Ag and Cu, in a certain degree also Ni, have antiseptic properties. Of those metals which were used for the experiments, Hg and Zn were also active, whilst Sn, Pb and Fe showed no effect. BEHRING wanted to determine whether it was merely a question of impeded bacteria growth, or whether these were actually killed. Therefore, after a few days, he proceeded to cut out such pieces of culture media as were apparently free from bacteria growth. He found that new cultures could not be obtained from such. The products of the bacterial metabolism dissolve metals. This also explains the varying effects of different metals on bacteria. The culture media round Ag becomes light brown. With Cu a blue tint appears after some time in Anthrax-cultures.

SPRINGER and SPRINGER jr, 1901, have studied the effect of copper sulphate on putrefactive bacteria. They demonstrated the bactericidal effect in a solution of egg white in water, in blood albu-

men, in meat, water and drain-water and their opinion is that these characteristics of copper sulphate may possibly be of therapeutic importance.

CLARK and GAGE, 1908, found that metallic Cu has a stronger bactericidal effect than Fe, Sn, Zn and Al, whereas CuSO_4 is weaker in this respect. *B. typhi* and *B. coli* live long in a solution of CuSO_4 1 : 100000, but they perish in a solution of 1 : 1000.

During the Great War, 1916, MESSERSCHMIDT tried to ascertain the bactericidal effect of various projectiles. Round a French copper-sheathed rifle bullet a bacteria free region formed itself in agar, whilst a German steel-clad bullet produced no bactericidal effect. With *B. pyocyaneum* one sees round the metal, after 24 hours, a light green region about $\frac{1}{2}$ to 1 cm. wide which turns violet later on. This is due to the activity of bacteria resulting in the formation of products, by the metabolism, which react with the metal in a colloidal medium. According to MESSERSCHMIDT this preventive effect is due to various copper compounds which form in the culture media through basic copper carbonate. The process begins with the formation of cupro compounds which then oxidize into cupri compounds. A certain bactericidal copper salt, probably copper lactate, was produced in the culture media even without the action of bacteria. Hyphomycetes are not influenced (*Mucor*, *Aspergillus*, *Penicillium*). Bacteria are scarce on copper things and coins which have been handled, owing to the fact that copper dissolves into salts the anions of which originate from sweat. In this connection it is chiefly a question of fatty acids, mainly butter acid. Strongly disinfectant salts of Cu, Sb, As, Zn, Mg and Pb form in the culture media. The effect of Ag, Cd, Bi, Mn and Ni is less pronounced and Au, Hg, Al, Sn, Fe, Pd and Pt are inactive. In the case of Cu the *B. pyocyaneum* perished after 3 days, *B. typhi* and *B. coli* after 2 to 3 and 4 days.

In 1919 SALUS has been experimenting with *B. coli*, *B. prodigiosum*, *Bac. mesentericus* and *B. paratyphi* A and he got the impression that the theory of a physical force as the cause of oligodynamic action can not be adhered to. Oligodynamic action sets in clearly only during dissociation. Ionization takes place. The process consists of an absorption of metal ions by bacteria and of gradual formation of certain complex compounds in the vicinity of which bacteria cannot exist. In 1920, whilst experimenting with silver coins and *B. coli*, *B. typhi* and *B. paratyphi*, SEIFFERT found a region with perished bacteria nearest to the coin; it was followed by one with

injured bacteria outside this another region still showing increased growth. At tests with *Staphylococcus* he even found the marginal elevation. *Vibrio cholerae* and *B. (typhi) murium* had no such. The culture media is also of importance; similar regions are not obtained on endo-agar. A region devoid of bacteria is not always necessarily followed by one showing increased growth. Oligodynamics are not specifically connected with metals, but the latter make it easy to demonstrate the action. A region seemingly destitute of bacteria actually contains dead ones. The evident bacteria free regions are understood by him as an expression of ARNDT's biological law: death, weakening, irritation.

In 1922 BUSCHKE and JAKOBSON studied the influence of Tl on *B. typhi*, *B. coli*, *B. dysenteriae*, Shiga-Kruse, Y, Flexner, *Vibrio Metschnikoff*, *Microc. pyogenes* and *Streptococcus*. In agar a bacteria free zone formed itself, 7 to 14 mm. wide, and outside this a marginal elevation with increased growth. The action of Tl cannot be explained as purely chemical. It is very probable that some physical forces act as well. During the experiments they made in 1925 together with KLOPSTOCK, Tl, Ag, Bi, Cu and Hg were used. Said authors maintained that these metals, influenced by the action of oxygen and carbon dioxide, formed soluble compounds, the oligodynamic action of which is caused by the presence of metals in the form of ions. If the ionization is hindered, the oligodynamic action is weakened or does not take place at all. Tl, when coated by paraffin, is inactive.

In 1924 FISCHER examined the effect of Ag, Hg, Pt, Cu, Sn, Pb, Zn, Ni and Fe on *B. dysenteriae* Kruse in agar cultures. Hg was strongly active, Ag proved weaker, and the action of Zn was the strongest. In certain cases a marginal elevation was observed, probably due to complicated chemical processes in the culture media. Different bacteria react differently with various metals. At tests with AgNO_3 , inorganic and organic acids being used, the following bacteria regions could be observed in the agar: dead bacteria, such with weak, increased and normal growths respectively. The concentration of hydrogen-ions plays an important rôle in this connection. The marginal elevation was at its highest with milk acid. Alkalies produced no such elevation, but the borderline towards the bacteria cultures was sharply defined. It is a question of solutions and diffusions and of reactions of certain chemical substances, which, however, have not been investigated in detail as yet.

FICKER found in 1898 that water in which copper coins had been kept for 24 hours killed *Vibrio cholerae* in about half an hour. Definite bactericidal action was found by him in copper sulphate diluted in a proportion of 1:50—60 millions. An addition of 0.001 per cent pepton prevented the action and it took two days to kill the vibriones. With 0.01 per cent pepton there was still a small quantity of vibriones alive after 10 days. Water, which had been 18 hours in the pipes, acted in such a way as not to leave any bacteria alive after 1 hour. Water, which had been 10 hours in the pipes, still showed a few colonies after 2 hours. In running water (through pipes) live vibriones were still found after 14 days. It is probable that water absorbs harmful particles from the metal of pipes.

In 1910 RANKIN found that if air is lead through water containing *B. coli*, the culture does not alter. If metals are added to the culture, e.g. Zn, quite a strong antibacterial action sets in. Cu and Al exercise no evident effect. If, by boiling, air is driven out of water before metals are added to it, the metals are not active. Thus the presence of oxygen is a necessity. A germicidal effect of still greater intensity may be obtained by leading air into the fluid containing metals. In this case too Zn yields the strongest activity. If there are sufficient quantities of metal in the water it can be sterilized. In the presence of Zn and Al the process is accompanied by the formation of H_2O_2 and also of metal hydrates. H_2O_2 alone cannot act in a bactericidal way, because the quantity thus formed is insufficient. A similar effect can be obtained by Cu alone without simultaneously forming H_2O_2 .

According to SCHLOSSBERGER, 1918, metals dissolve in the culture media in minute quantities, Cu slightly more than Ag, the former having also a more pronounced effect. Bacteria absorb metal particles and they either die thereof, or their development is impeded.

STRECK, 1919, points out that it has been possible to observe two different expressions of the oligodynamic action, viz.: germicidal action in water and the checking of bacteria in agar or gelatine cultures. He worked principally with *B. (typhi) murium* and copper foils in water, but he has also made experiments with *B. septicaemiae* and *B. pyocyaneum*. The oligodynamic action of copper depends upon the proportion of its surface to the quantity of water. The latter, if increased, diminishes the effect. In quantities of 10—1000 c. c. water there is a definite relation between the metal surface and the volume of water, taking into consideration the time necessary to kill bacteria

which usually lasts from 18 to 28 hours. In «activated» glass vessels the action of copper sets in sooner. Whilst a copper foil of 0.45 cm^2 in 10 c. c. water killed *B. typhi* in 24 hours, the same surface of copper in an «activated» vessel proved to be deadly within 8 hours. He further found that water which is given oligodynamic properties by copper, «activates» both the original vessel and the following one.

DOERR, 1920—22, in partial collaboration with BERGER found that water, activated by Ag, shows all the qualities of a disinfectant. The bactericidal properties vary according to whether it is being dissolved with distilled water, or whether it is boiled until evaporation occurs. NaCl weakens the effect and horse-serum stops it altogether. Heating to a glow, boiling and bedding in agar checks the action, whilst treatment with diluted acid restores it. The active substance can be dialyzed. Everything seems to point to the fact that there is a formation of cytotoxic compounds soluble in water either through the oxygen in the air, or through H-ions. «Inactivated» silver can become «activated» by prolonged exposure to air. In CO_2 or O_2 the process is faster. In all probability Ag_2O is the bearer of oligodynamic action. When KCN is added both silver surfaces and silver-activated water lose their bactericidal properties.

According to LAUBENHEIMER, 1921, the exact nature of oligodynamics has not been fully explained as yet and this question has been further complicated by SAXL's theory regarding a physical power which exists on metal surfaces and originates from the air. His own opinion is that the metal, Ag, has dissolved in the culture media.

According to SORDELLI and WERNICKE, 1921, Cu does not activate distilled water in the presence of H_2 , but it does so in the presence of O_2 and CO_2 . The metal dissolves and there is a parallel between its solubility and oligodynamic action. O_2 is an absolute necessity for dissolving Cu, but, alone, it is insufficient and CO_2 must be used as well. The bactericidal action of water thus activated was tested on *B. paratyphi* A.

WERNICKE, DORTZENBACH and DE LA BARBERA, 1927, found that pure metallic silver is insoluble in distilled water in the presence of H_2 . On the other hand it dissolves in the presence of either O_2 or a mixture of O_2 and CO_2 ; the water acquires thereby oligodynamic properties which have been tested on *B. paratyphi* B.

WERNICKE and MODERN were able to ascertain in 1929 that distilled water dissolves Ag when in touch with it, acquires oligodynamic properties and contains Ag in the shape of ions to the extent

of 0.00005 mg. to the c. c. If this water becomes exposed to electrolysis, it completely loses its oligodynamic value and the metal is being precipitated onto the cathode. Activation of water thus depends on an ionized solution of the metal. The germicidal property of Ag- and Cu-solutions is preserved even in the presence of H_2 without O_2 . It is therefore reasonable to suppose that an ion is poisonous in itself. Oxygen is only necessary when activating water by contact with metal, because the latter then dissolves more readily.

KUROKAWA, 1925, examined the bactericidal properties of Ag, Al, Au, Cu, Mg, Fe and Zn in connection with various bacteria. These metals were used in powder form, partly in water, partly in a 0.85 per cent solution of NaCl, in 0.5 per cent suspension, Au in 0.1 per cent. Ag acted germicidal in 22 hours, Mg and Au after 4 days. A solution of NaCl increased the action of Cu and slightly of Ag, but did not affect the other metals. They affect various bacteria differently. Even if diluted up to 1:32, an Al suspension is still deadly for *Streptococcus*, but it affects but mildly *B. typhi* and *B. dysenteriae*. Mg acts comparatively strongly on *B. typhi* and *B. paratyphi* A, whereas its action on *B. paratyphi* B. and *B. coli* is weak. Au affects *Streptococcus* more than *B. paratyphi* B. Cu influences *B. coli* less than *B. paratyphi* A and *B. The effect of Ag on Staphylococcus and B. paratyphi* A was similar in both cases. According to KUROKAWA Au is the most powerful in action because of its small quantity. Then follow Cu, Ag, Al and Mg. The effect of Zn was the weakest of all and Fe was totally devoid of oligodynamic properties.

ROSENKRANZ studied in 1920 the effect of copper chloride, copper nitrate, copper lactate and copper acetate on various bacteria. *B. (typhi) murium* was killed by every salt within 24 hours in a solution 1:10000. In weaker solutions copper chloride is the most active of all. *Staphylococcus* offered somewhat more resistance. Copper chloride killed within 24 hours even *B. prodigiosum*, *B. pyocyaneum* and *Vibrio Metschnikoff*. Of *B. septicaemiae haemorrhagicae*, *Proteus vulgaris*, *B. subtilis*, *B. mesentericus*, *Sarcina lutea* and *Sarcina rosea* only a very small quantity was alive still after 24 hours. Organic substances damped the action of copper salts, whereas ammonium sulphide stopped it entirely. The same thing happens if ammonium sulphide is added to water, in which copper foil had been submerged during 24 hours, when small quantities of copper are found to exist in the water. According to MITTELBACH, 1921, soluble copper salts are endowed with strongly disinfectant qualities, which

stand in proportion to the Cu-content. Various kinds of bacteria react differently. CuCl_2 is the most active of the copper salts. The effect of salt is weakened in a medium containing albumen. v. LINDEN has arrived at the same results.

SÜPFLE, 1920—29, understands the oligodynamic action of metals and certain chemical substances as the outcome of their capacity to dissolve in the culture media. One cannot speak of the difference between oligodynamic and chemical action, as it is the question only of a different intensity. In both cases, as far as quantity goes, the action of substances is limited and can be stopped altogether. With other words: a given quantity can kill or impede the development of a definite quantity of bacteria. Organic substances decrease the oligodynamic capacity of Cu, as shown already by v. NÄGELI. The generation of oligodynamic properties of metals or metallic salts in each separate case necessarily requires a solvent. A metal can even be dissolved in agar, but in a small measure only. The dissolution and diffusion is not so marked in comparison with the same process in water. Many different circumstances exercise their influence on the process, viz.: the concentration of agar, age, temperature, coagulation time and substances which had been added. After one day has elapsed the diffusion may be considered complete. It is more intense in 1 per cent than in 3 per cent agar. The region which is devoid of bacteria is also larger in 1 per cent agar. There is no bacteria growth as far as the poisonous effect reaches. The border of the culture can have different aspects; it depends on the substance and bacteria used. In regard to poisons it was found that the stronger the concentration of poison, the fewer and weaker were the bacteria. With less poison the colonies grew larger and more numerous; in some cases they were even richer than the control colonies. In the case of certain substances no difference was observed when a weaker concentration was used. In all these differently resulting tests it is probably a question of an activity optimum of the substance in so far as it attacks two places of the bacteria protoplasm: the life-intensifying and life-impeding one. If these two parts are sensitive in a different degree towards the poison and are given a certain concentration, an optimal action takes place. According to SÜPFLE the biological law of ARNDT-SCHULZ is not always valid. He showed in 1929 that when oxygen is lead through collargol before being added to the agar, the same effect is obtained on bacteria independently of whether the test conditions are anaerobic or aerobic. The oxygen

causes ionization in collargol. Under the influence of oxygen ionization takes place even in the case of colloidal gold. The facts disclosed by a careful analysis in regard to the process of oxygenous action do not contradict the chemical perception of oligodynamics, on the contrary: they support the theory. The oligodynamic action is a function of metal ions.

When using certain chemical compounds whilst experimenting along the lines of ARNDT-SCHULZ's biological law, HOFMANN, 1922, found the law confirmed. On the other hand the use of some other substances failed to produce the same result. In 1929 he examined the effect of collargol on *B. pneumoniae* Friedländer under different partial pressure of oxygen. Silver oxygen compounds are produced thereby then follows their ionization, resulting in oligodynamic action. CO_2 has the same effect as oxygen. Bacteria can stand 500 times as much collargol in the absence of oxygen as without it. The more the quantity of oxygen is increased, the less concentration is required in collargol. Bacteria withstood a greater concentration of Au and Cu in the agar in the same way as was the case with collargol. Oligodynamic action could be explained as the function of metal ions. The ionization of pure metals takes place as soon as the oxygen reaches them. Metal oxygen compounds are formed which then ionize. When metallic salts are used the results of the tests are the same whether oxygen be present, or not. In regard to the nature of oligodynamic action HOFMANN confirms the chemical theory.

HERZBERG, 1923, is of the opinion that oligodynamic action is due to the oxygen which becomes densified by metal ions. The bactericidal action of Cu, Hg and collargol can be divided into two phases: the action of metal poison and that of oxygen. After the metal ions have become absorbed by the cell wall oligodynamic action sets in owing to the effect of oxygen alone, whilst the metal ions only act conductively. Collargol causes quite a strong condensation of oxygen in agar. HgCl_2 is somewhat weaker and Cu is the weakest. The action of oxygen is probably catalytic.

MUNN and HOPKINS, 1925, have studied the effect of the following tellurium compounds on *B. coli*, *B. typhi*, *Staphylococcus aureus* and *B. anthracis*: acid tellurtartrate, diethyl-ammonium-tellur-chloride, double iodine salt and silver-ammonium-tellurite. They found the following to be active in the absence of organic substances: tellurtartrate against *B. coli* and *B. typhi*, in a lesser degree against *Staphylococcus aureus*. Diethyl-ammonium-tellur-chloride affected *B. coli*.

Double iodine salts affected all bacteria. The effect of silver-ammonium-tellurite was the strongest and may be compared with that of silver nitrate. *B. anthracis* was influenced more slightly by silver-ammonium-tellurite than by silver nitrate. The action of double iodine salt was the weakest.

BAIL, 1919, found that Gram-positive bacteria were more apt to be influenced by metals than Gram-negative bacteria. During experiments with different metallic salts in varying, very weak concentrations TAUCHERT found in 1931 that the difference between Gram-positive and Gram-negative bacteria became evident only when metals with three valencies were concerned. The Gram-positive bacteria were more resistant. Some connection between the valency of metals in general and its killing effect could not be established. MURTO, 1927, has not noticed any difference in this respect.

ANDRESEN, 1927—28, found that in a culture media containing pepton and keeping its concentration of silver ions unchanged during 48 hours, a silver ion concentration of $0.16\text{--}0.6 \times 10^{-11}$ impedes the growth of *B. coli* and *B. typhi*, provided there is sufficient non-dissociated silver to upkeep the concentration of ions. The acting agent is the silver ion.

COOPER and NICHOLAS, 1930, found that Cd-compounds have a greater influence on bacteria than Pb-compounds, but are nevertheless weaker than Hg-compounds. Cadmium carbonate and -sulphide give constant colloidal solutions, phosphate- and molybdate salts give less constant solutions. With lead the case is to the contrary. Complex compounds of sodium-pyrophosphate and Cd and Hg with two valencies produce a strong bactericidal effect, the former in a concentration of $1 : 10^6$, the latter in $1 : 10^4$.

TÜRKHEIM, 1929, noticed oligodynamic action of some metals in agar cultures. Of all the metals used in the tests Cd proved to be the strongest. Then followed a group of Ag, brass, Cu, Hg and finally, gold, silver, copper and brass coins. Ag- and Cu-amalgamate were but slightly effective. Of all the bacteria *Staphylococcus albus* offered the best resistance, followed by *Streptococcus* and *Acidobacterium lactis*. Observation showed that bacteria became gradually used to the metals which became less effective in the end. It is probable that the metals shed small particles of themselves.

LA CAVA, 1931, objects to the expression »oligodynamic» powers. The phenomenon shows two phases: the element dissolves in the culture media and then the germicidal action of the element sets in.

A metal may be dissolved, but owing to its weak bactericidal influence the growth of bacteria is unimpeded. Cu and Ag, dissolve comparatively easily and, being considerably poisonous, possess a high bactericidal power. The germicidal action of silver is generated by silver oxid detaching itself from the surface of the metal and by its subsequent colloidal state in the culture media. The more silver oxid becomes detached, the more pronounced grows this property. If the oxid be removed from the metal surface by heating it to a glow, the antibacterial action ceases. Silver oxid, whilst in colloidal form, produces an ion-compound with agar colloid, thus altering the culture media in a way which is harmful to bacteria.

EICHBAUM, 1932, uses a solution of 100 mg; Ag_2O in 100 c.c. water. After centrifugalization the solution is brought up to 1 : 50. This constitutes a standard solution and contains 480 gamma silver per litre. In a double dilution = 240 gamma per litre this solution destroys 3—5000 coli bacteria per 1 c.c. in 2 hours. The effect of oligodynamic silver water can be defined only by biological tests. Chemical definition of quantity or electrometric measuring of ions in the case of silver cannot show in every single instance the character of the biological effect of oligodynamic water.

GUTSTEIN'S, 1932, point of view is that the poisonous properties of salt solutions of heavy metals depend on their affinity to the lipoids of the cell membrane. He was able to show by experiments a complete accord between the poisonous quality of metallic salt solutions and the precipitating property of their actions against a phosphatide prepared on beans. The decrease of the precipitating property was in conformity with the series Hg-Ag-Zn-Cu-Ba-Ca.

PIORKOWSKI, 1932, states that silver tooth-filling pegs in vitro are able to destroy various kinds of bacteria and that this bactericidal effect can be intensified by exposing the silver peg to a 3 per cent solution of H_2O_2 during a period of 12—24 hours. TREBITSCH, 1932, too points out the importance of silver as tooth-filling material because of its oligodynamic effect.

LASSEUR, PIERRET, DUPAIX and MAGNITOT, 1932, have studied the bactericidal effect of a silver wire of 0.2—0.5 mm. ϕ in different solutions with a pH of 6.5—7.46 and they found that the shape of the wire is of no consequence when the experiment last during a longer period.

According to LÖHNER, 1917, the region which forms itself round metals on the agar is caused by small quantities of dissolved metal.

The conditions of solution and diffusion are quite complicated in agar. The constancy of the free region depends on the diffusion being completed at the time the bacteria begin to develop. If metals are still in the state diffusion when the colonies have begun to develop, the growth is partly impeded and the border is not sharp. The increased growth in the border zone is caused by the fact that the concentration of metal is weak and only irritates the bacteria.

BAUMGARTEN and LUGER, 1918, are of the opinion that the marginal elevation is caused by small quantities of bacteria poison which irritate the bacteria. According to LUGER, 1920, the borderline between the colonies and the region which is devoid of bacteria is not always sharp. This does not depend on the kind of bacteria used, but is due to the physical and chemical condition of the culture media. In gelatine the border line is seldom sharply defined.

COBET and VAN DER REIS examined in 1922 the effect of arsenious acid on *B. prodigiosum*, *Staphylococcus aureus* and *albus* and *B. coli* in petri dishes. They found the zone nearest the compound to be devoid of bacteria. Outside this there was a region of increased bacteria growth and then followed a zone in which bacteria had their usual appearance. These different stages depend on favourable conditions prevailing in the culture media, i.e. not on the irritation of a certain concentration of poison. Whilst experimenting with *B. coli* in 1922, SEIFFERT came to the conclusion that the increased growth of bacteria along the border of the colonies is due to favourable conditions in the culture media and also on the oligodynamic action of metals. He considers the existence of a marginal elevation as a special case of ARNDT's biological law. According to SCHUMACHER, 1922, the oligodynamics of silver are but a question of active silver ions. This explanation, however, is insufficient when discussing the presence of LÖHNER's so-called marginal elevation. The formation of it is caused by ions which are active as oxygen catalyzers. This oxidizing effect stimulates the life of bacteria and the colonies grow larger, but without increasing in number. Furthermore, sufficient culture media is required, and as the marginal elevation is found exactly in the borderline of the free zone, this circumstance is to be reckoned with as well. It is therefore a question of two phases: oxygen transferring ions and good nourishment possibilities. SCHNABEL, 1922, says that the further from the metal the weaker is the concentration of ions in

agar. The profuse growth in the border zone is in complete accord with ARNDT-SCHULZ's biological law. The causes governing a double marginal elevation in agar cultures were first mentioned by DOERR, and a more detailed description by MURTO is found in chapter 5.

CHAPTER 4.

The effect of metals and chemical compounds in regard to fungi and protozoa.

According to BOKORNY, 1906, 0.001—0.0025 gr. copper sulphate and 0.005—0.01 gr. bichloride of mercury have a deadly effect on 10 gr. yeast-cells. 0.1 gr. lead acetate, 0.05 gr. ferrous sulphate, 0.3 gr. cobalt nitrate and 0.2 gr. nickel sulphate produce the same effect, whereas 0.4 gr. manganous sulphate is unable to kill 10 gr. yeast. Of all the metals in this group Fe was most highly effective. Mn proved weak, although its atomic number and weight is very close to that of the foregoing group. The strongest effect was produced by Cu. *Spirogyra* were destroyed by solutions of both copper sulphate 1 : 10^6 and bichloride of mercury 1 : 10^8 . Cu proved weaker in this case.

French scientists have paid special attention to the study of the effect of small quantities of metal on *Aspergillus niger*.

SAUTON found in 1911 that the fungus in the absence of Fe, does not form spores in RAULIN's solution, i. e. when Zn is absent as well. Spores cannot form in the presence of ferrous citrate, but they do so in the presence of ferrous sulphate. He is of the opinion that Fe is not a necessity to the formation of spores on *Aspergillus niger*; the formation of spores is stimulated by some substance, possibly a compound, contained in ferrous sulphate, but not found in ferrous citrate.

MILLE ROBERT, 1911, came to the conclusion that Ca does not exercise any serious effect on the development of *Aspergillus niger*, at least not in the concentration she used, viz. 1,20th mg. to the litre. However, the fungus binds Ca completely when in small quantities and only partially when in larger ones.

BERTRAND and JAVILLIER found in 1912 that Mn and Zn stimulated the growth of *Aspergillus niger*. A greater quantity Mn is absorbed in the presence of Zn than in its absence. The development

stands in proportion to the quantity of Mn in the culture media. The formation of gonidia is also influenced. The increase of a culture inoculated on media containing manganese does not solely depend on the manganese in the substrate, but it is also due to the quantity absorbed by the cell where it influences the assimilation of nourishment.

JAVILLIER studied in 1912 whether certain elements could influence *Aspergillus niger* to grow in the same way as when under the influence of Zn. He found that in a concentration of $1:10^5$, $1:10^7$, which allows Zn to act favourably, the following elements lack effect: Na, Os, Ca, Ba, Th, rare kinds of earthy metals; U, Ti, Pb, Cu, Hg, Pt, Ag, Cs, Ph, Cl, Br, As, Sb, Te, V, Bo, Ti, Zr, Sn. Other elements, like Fl, I, Rb, Li, Sr, Al, Co, Ni, Cr, Mo, Tu, Au, Pd, only in a certain degree influence the increase of growth, whilst Bi and Se proved poisonous. Only one element, viz. Cd, showed the same properties as Zn, although in a slightly weaker form. This is explained by Cd's position in the periodic system where it stands in the same group as Zn, which latter has quite a specific physiological effect on *Aspergillus niger*. He also points out that glass vessels, sheltering cultures, could yield Zn from the glass itself and thus prove misleading. By substituting Cu for Zn in a culture media he found that in a quantity of $1:10000$ Cu increases the growth of *Aspergillus*. U has no visible effect. V, when in the form of sulphate in a concentration of $1:500$, increases the growth by 40 per cent. To obtain the same effect greater quantities of Cu and V are required than of Zn.

JAVILLIER and TSCHERNOROUTZKY, 1913, have studied the effect of Zn, Cd and Be on different Hyphomycetes and have arrived at the conclusion that the growth of *Paecilomyces varioti* is greatly increased by Zn in weak concentrations, such as $1:1$ million or $1:5$ millions. Cd is weaker and Be yields no effect at all. Two kinds of *Penicillium* (*P. caseiolum* and *P. glaucum*) are still influenced by Zn in a concentration of $1:100$ millions. Cd affects the former, but is rather toxic in larger doses. The latter is affected by Cd but in a small measure and quickly gets toxic in stronger concentrations. Be is without effect.

BORNARD, 1913, found that *Aspergillus niger* in RAULIN's solution in a silver vessel shows slower development of the mycelia, the sporulation nevertheless is not checked. In spite of frequent transplantation the fungus could not be induced to develop normally in a silver vessel. After 10 days of contact a chemical analysis of the fluid

could not disclose Ag. If cells are cultivated in vessels made of the following metals Al, Pb, Zn, Cu, Ni, Fe, Sn, Pt, Hg, their development is prevented by the vessels themselves with the exception of Al and Pt which do not dissolve. The development of cultures does not take place above Hg.

FROUIN and MERCIER, 1914, found that a solution of sodium vanadate 1 : 100000—1 : 2500 stimulates the growth of *Aspergillus niger* and increases the formation of spores. BERTRAND, 1912, has shown the important rôle played by Mn in the formation of *Aspergillus* spores, even in the presence of Zn and Fe. He tested the effect of minute quantities of Mn on the fungus, viz.: 1 : 10^8 , 1 : 10^9 and 1 : 10^{10} . The result showed that cultures with Mn were larger as compared with control cultures without Mn. In order to avoid a possible reaction between the metals and the glass the tests were made in quartz vessels. In 1914 he found that silver nitrate in a concentration of one gramme-molecule to a million litres, $M/10^6$, damaged *Aspergillus niger*, but had no visible effect in a concentration of $M/10^{22}$. Such organisms as are sensitive to certain elements are greatly influenced by even the smallest quantities of the latter. LEPIERRE, 1913, substituted Zn in RAULIN's solution by U- and Cd-salts. U is bound by the cell; growth and formation of spores does not increase and may even be prevented by use of the U-salt in too high a concentration.

SPIRO, 1915, used chemically pure copper and yeast-cultures in his tests. Copper dissolves more easily in the presence of oxygen. The dissolved copper is oxidized by a substance from the cell body and is bound by cell constituents. When Cu has disappeared from the solution, a new supply of copper can be dissolved therein. Cu is absorbed by surface action and the absorption causes a chemical reaction. Both the oligodynamic and the chemical action are of the same kind; the difference lies in quantity and shows varying elapsed periods of time. A bacteria with its complicated structure reacts differently in concentrated solutions than in diluted. It is difficult to state with exactitude where the chemical action ceases and where the oligodynamic one sets in.

NĚMEC and KÁŠ studied in 1920 the effect of Se on some kinds of *Penicillium*. They used 1 gr. sodium selenite in the following solutions: 1 : 10^9 , 1 : 10^8 , 1 : 10^7 , 1 : 10^6 , 1 : 10^5 and 1 : 10^4 and found that small quantities of Se increased the growth of *Penicillium candidum* and *Penicillium Roqueforti*, even in the presence of Zn and Mn, the former

being somewhat more sensitive. In cases where Se stimulates the growth, the circulation of mineral matter increases and the total quantity of ash is greater than in cases where Se is injurious.

HANNA LAPPALAINEN showed in 1919 that minute quantities of Zn, viz.: 0,000005 per cent to 0,0005 per cent, cause variation in *Aspergillus niger*.

ZERNER and HAMBURGER, 1921, have studied the effect of AgCl, AgNO₃ and metallic silver on yeast and found that metallic silver yielded no effect at all; the easily soluble Ag₂CO₃ is more poisonous than AgCl which does not dissolve so readily. Should the solubility of the above mentioned compounds be decreased by adding suitable salts, their poisonous action becomes likewise weaker. They are of the opinion that the silver and the albumen of bacteria form a complex salt.

MARBOE studied in 1930 the effect of bright metals on yeast. The specific effect of metals is different: Cu, Ag, Cd, Hg, Cr were highly effective; the action of Zn, Al, Sn, Pb, Ni was weaker; Mg, Bi, Mn and Fe were but indifferently effective. The actual effect of metals depends on their specific effect in connection with their solubility. Small quantities of metals act as irritants on yeast and shorten the period of generation, whereas in higher concentrations their effect is to the contrary. The effect of metals is reduced when boiling the media. If there is a bright metal present at the same time, the effect ceased or grew weaker in accordance with the characteristics of this particular metal. The action of metals is caused partly by the poisonous effect of metallic salts and partly also by oxidation and reduction processes in the culture media.

SCHWARTZ and STEINHARDT, 1931, studied the absorption of copper by the mycelia in a culture of *Aspergillus niger* van Tieghem in different oligodynamically active copper concentrations between 10⁻² per cent and 10⁻⁷ per cent. The growth curve with copper all the time lies under that where the metal is missing. The quantity of copper absorbed by the mycelia during a given period of times is, to begin with, rather independent of the copper concentration in the solution, viz.: the maximum amount of copper is shed during the first days and later on both the absolute and the relative quantity of copper is reduced.

V. ANGERER, 1922, has tested the effect of Cu and Ag on water-spirochaeta. His argument was that organisms, which do not succumb to the effect of a brass tap when coming in touch with it, are

also more resistant to the oligodynamic influence of metals. He introduced copper and silver bars into sterilized pipe water, put water-spirochaeta in some vessels and *Vibrio Metschnikoff* in others. In the presence of Ag the development of water spirochaeta proceeded unhampered; the presence of Cu reduced it noticeably within 5 days, and after a fortnight no micro-organisms were to be found. *Vibrio Metschnikoff* could not be detected the day after it first came in touch with the metal-activated water.

BEGER, 1923, added copper foil to 10 per cent of rabbit serum cultures of WEIL's spirochaeta. Samples were taken after 5, 10, 20, 30 and 60 minutes and thereafter at intervals of one hour each. Investigations showed that after 2 hours the movements of the spirochaeta became slow, but few moved after 5 hours and after 6 to 7 hours all movement ceased completely.

SHIGA, 1924, worked with 2 Weil strains, *Spirochaeta icterogenes* AW and W 10 and 2 water-pipe strains; *Spirochaeta pseudoicterogenes* Berlin (Rg V) and Erlangen, E. The following metals: Cu, Pb, Hg, Fe and brass were used, either in finely cut bits or in the form of powder, with the exception of Hg. The spirochaeta were added after 24 hours. In some tests the metals were added to richly developed cultures. In the first series the water spirochaeta began to develop in the same way as in the standard culture devoid of metal. 7 days produced no changes; in cultures with Hg only most micro-organisms were motionless and after 3 weeks but a few degenerated remains of them were to be found. After a similar period of time Cu had also affected chiefly the Berlin strain. Fe, Pb and brass failed to produce any effect.

On the other hand but 2 days sufficed for Cu, Hg and even Fe to destroy the Weil spirochaeta. The effect of Pb and brass during a period of 2 to 3 days left but a few straggling micro-organisms which soon disappeared altogether. In the second series, where metals were introduced into rich cultures, the results were the same. This showed that water strains were much more resistant in respect of certain metals, which also explains their existence on brass taps. Tests with colloidal metals, Cu, Ag, Pb and Se confirmed the greater resistance of water strains. Colloidal Ag failed to affect them. SHIGA finds this resistance very noticeable, but in the case of bacteria it is not so marked.

ANJOW, 1928, chiefly followed the method of SHIGA. He tested the effect of Cu, Hg, Pb and Fe on water-pipe strains, Erlangen and Tokio.

and 9 different Weil strains. After 20 days Cu still had no effect on the water-pipe strains, whilst the Weil strains, with but a few exceptions, were destroyed in 3 days. The same effect was obtained by Hg. Pb and Fe could but injure a few Weil strains after a lengthy period of exposure. Pb and Fe exercised a slightly better effect on a certain Weil strain (*Leptospira akiyama*). Colloidal silver containing 0.06 per cent silver, colloidal selenium containing 0.02 per cent selenium in different solutions like 1 : 50, 1 : 100, 1 : 250 and 1 : 500 effected pure metals similarly. The pathogenic strains lived 4 to 5 days, whilst water strains were still alive after 28 days.

UHLENHUTH and FROMME, 1931, have also found that Weil strains were sensitive to the oligodynamic action of copper, whilst water strains proved resistant in the face of this action.

HIRSCH, 1931, studied the resistance exercised on copper by spirochaeta of the Weil-type and by water spirochaeta. He used: water-pipe strains Erlangen, Freiburg and Rg; natural water strains Leiden, Amsterdam, Tjikini and Parapatem I; Weil strains Berlin RGA, Franken, Schotte, Elte, Banghinan, 221; rat strains Oudekerk I, 18, Abbattoir VIII; a hebdomadis strain and a marsh-fever strain Hamburg. The Spirochaeta were bred in 10 per cent rabbitserum-water; 50 c.c. serum was allowed to be influenced by 1 gr. copper during 40 hours at 58° C, whereupon the liquid was submitted to centrifugalization and different solutions were made out of it. Water strains proved much more resistant than Weil strains. The former could be divided into two groups: the pipe-water strains Erlangen, Freiburg and Rg which showed great resistance, and; the natural water strains Tjikini, Parapatem I, Leiden and Amsterdam which proved 10 to 20 times more sensitive. The action of the tested Weil strains was similar to those of the natural water strains which were sensitive to metals, only the strains hebdomadis and Berlin RGA were somewhat more resistant. HIRSCH chiefly confirms the results obtained earlier by other scientists.

LÖHNER and MARKOVITS, 1922, studied the oligodynamic effect of copper on paramaecia. In order to avoid the influence of colloids and other substances possibly contained in ordinary water which could bind the metals, he used the latter in distilled water, which proved to be perfectly harmless to micro-organisms during 20 hours. With »copper-water» it was observed at first that an irritation and an increased liveliness of the paramaecia, up to 25 per cent, had

taken place. After different periods of time their movements become slower and finally they die and sink to the bottom. The explanation lies in the fact that metal is being absorbed by the living cell. An increase in quantity of paramaecia in the fluid necessitates a longer period of time for their destruction.

CHAPTER 5.

The physical power of elements and their radiation in regard to oligodynamics.

THIELE and WOLF, 1899, found that the effect of Ag on bacteria agar cultures increases when the metal outside the culture media is put in contact with electronegative metals like Pd, Pt and Au, or with C. In similar tests metals like Fe, Al, Zn and Mg, highly charged positively, influence such metals into action as are otherwise unable to produce the effect. The increased effect of Ag, however, is caused by its solubility.

Since 1917 SAXL had written a series of articles, dealing in detail with various oligodynamic phenomena. In 1924 he published them all in the form of a monography. He has tested the bactericidal properties of metals and metallic salts, applying them to the sterilization of drinking water, to therapeutics and to the preparation of vaccines. His opinion on the nature of oligodynamics differs from that of other scientists.

According to SAXL oligodynamic action of metals and metallic salts is mainly of a biological character. When it sets in such a dissolution of minute quantities of metal or metallic salt takes place, that either the chemical effect of the dissolved substance cannot be shown, or else its intensity is so weak that the solution must be described as having but a very weak chemical action. The oligodynamic problem culminates in the question quoted below. On what does it depend that metals or metallic salts which dissolve in such minute quantities as to be chemically inactive, or nearly so, still have a definite biological effect? He has also found that different metals act differently on various bacteria. The oligodynamic action does not effect bacteria directly, but it causes changes in the culture media. The effect can be transferred to other matters. Water, physiologic NaCl solution, glass, gelatine and agar acquire oligodynamic properties from contact with copper or silver. SAXL summarizes his opinion about

oligodynamics as follows: the so called oligodynamic characteristics of metals are nothing but a peculiar form of bactericidal power which cannot be explained in a purely chemical way, but which may be possibly due to a physical phenomenon taking place on the surface of metals and may be transferred to other substances and media; it should be regarded as a far reaching force originating from the air and still unknown as to its nature. He fails to give a satisfactory explanation of oligodynamics. Most scientists embrace the theory of the dissolution of metals, but SAXL contradicts them all by saying that they have not proved by oligodynamic tests the presence of silver in the form of ions.

RUETE, 1925, in his convictions stands nearest to SAXL. Finely distributed metallic Ag and Au were allowed, in the course of 24 hours, to be in contact with 10 c. c. water. The metal thereupon was taken out of the water which he inoculated with 3 normal loopfulls *Staphylococci*, *B. coli*, *B. paratyphi* etc. An agar culture from these metal-water-cultures showed prevented or destroyed the growth of bacteria. It could not be proved that the glass was activated. Ag was more strongly active. There was no effect if the quantity of bacteria was large. Full sterility was reached when the metals were left a long time in water in a proportion 1—2—4:1000. RUETE is of the opinion that it can not be a question of a purely chemical solution; a power emanating from the metals is rather responsible for the effect. In all probability there are also some physical phenomena which play a rôle in this respect.

PFAB, 1928, is also sharing SAXL's opinion. The influence of Zn in an agar culture is due to this metal's radiation which very much resembles a radioactive one. When developing photographs taken of these cultures he noticed a peculiar phosphorescence round Zn which was more marked, if Zn had been exposed to sunlight. When Zn was left exposed to sunlight for some time and then put on a photographic plate and the whole wrapped up in black paper, an ensuing development of the plate showed a picture of this piece of metal. Owing to its bactericidal property PFAB has used silver foil when treating burns (1930).

ÖHOLM, 1909, has studied the radiation from metals. He used Zn, Cu, Mg, Ag, Ni, Co, Fe, Al, Pt, Au, Cd, Bi, and Pd, placing them in a photographic plate-box within 2 mm. from the plate. The blackening of the developed plates showed different grades in regard to these metals' intensity of action. The activity of Zn was strongest. Higher temperature produced a corresponding increase in the effect of

metals. If such substances, which are not gas-tight, are placed between the metals and the plate, the action of the metals is unimpeded, glass prevents the action of the metals, so does also a current of air lead through the plate-box. The blackening of the plates is not caused by rays, it is due to a gas which, in the case of easily oxidized metals, consists of H_2O_2 . Where precious metals are concerned the chief acting agent may be oxygen, occluded by the metals. It is therefore anything but a question of radiation from metals.

A. BECKER, 1925, is also of the opinion that, contrary to what was formerly presumed, a photographic plate is in many cases not blackened at all by the radiation from metals; it is rather a question of the influence of chemical substances, e.g. of H_2O_2 which develops comparatively easily on many freshly split metal surfaces and may cause the blackening of a photographic plate.

V. NEERGAARD, 1925, has tried to investigate whether Ag could be found in the form of ions in oligodynamically active silver-water, or whether it was a question of physicalic action, as interpreted by SAXL. The Ag-ion is the actual bearer of oligodynamic action. V. NEERGAARD confirms DOERR's theory, viz. that oligodynamic action is due to a soluble metallic salt compound adhering to the silver surface, and which can even be ascertained as to quantity. He finds nothing to support the theory of a physical power on the surface of the metal.

In 1927 MURTO made extensive oligodynamic experiments. His tests were made in petri dishes on a rotating table, bacteria were spread thinly on the agar by means of a wide platinum triangle. The result of the resistance of bacteria to the oligodynamic influence of metals is due to zooglea. The more zooglea a bacteria contains, the greater resistance it can offer. Generally speaking, Coccaceae are resistant, Bacteriaceae are sensitive, as is also the *Proteus* group, rather sensitive are Spirillaceae, e.g. Vibriones; still more sensitive-ness is shown by Corynebacteria, e.g. Corynebact. diphtheriae and pseudodiphtheriticum, Mycobacteria are the most sensitive of all, when with Sb. Higher fungi are resistant; a retarding effect can still be noticed with *Saccharomyces ruber*. *B. paratyphi* is influenced less than *B. typhi* and *B. coli*. Amongst vibriones the pathogenic kinds are particularly sensitive. The effect produced by both Ag and Sb proved to be the same, whether they were heated or not. Cd effected *B. pyocyaneum* more strongly when heated. Diluted mineral acids increase in a certain degree the oligodynamic properties of elements,

but the inactivation and regeneration of metals is not a characteristic peculiar to oligodynamics. The importance of Cl, if any, is of no consequence. Oligodynamic action under the lid propagates through the air, which spreads metal particles. Every metal effects in its own specific way various bacteria, to quantity as well as to quality. One can not generalize metals into active and inactive, as there is hardly a metal which effects similarly all kinds of bacteria. Certain regularities may be noted in the periodic system of elements, viz.: b-metals only produce injurious effects, whereas a-metals promote the growth of bacteria. Injurious metals of the periodic group may be classified into certain groups. The acting metals in group I are Cu, Ag and Au; in group II Zn, Cd Hg; in group III In; in group IV Sn and Pb; in group V As, Sb and Bi. MURTO has drawn up so-called metal charts for different bacteria. The injurious oligodynamic effect is first of all growth impeding. According to MURTO there are thus two kinds of oligodynamic action: a harmful and a promoting one. Should elements be inserted into БОHR's table, the harmful metals will be the following: 27—34 in column IV, 45—52 in column V and 77—84 in column VI. Harmful metals are found under the lines downward from Mg, whilst the promoting metals are above them. By the position of Mg in this system one feels inclined to expect that it has both the properties in question. This in fact was proved by MURTO in regard to *B. fulvum*.

Hg and Sb affect *Mycobacteria* even if the metals on the agar are in a glass vessel which, in the case of Sb-powder, should have walls not exceeding 20 mm. in height. Although there is evidently a zone round the harmful metals which is free from bacteria, they are able, nevertheless, to live in it quite a long time. Thus inside a vessel with a lid screwed down *B. pyocyaneum* kept alive for 6 months together with Sb.

MURTO's conception of oligodynamics is founded on the theory about electrons. The specific qualities of an element are shown by its protons. The oligodynamic action takes place through the intermediary action of atoms. Harmful action is produced by the positively charged atom which, at the time, has a lesser amount of electrons, promoting action by the negatively charged one which is temporarily in excess of electrons. The destruction of bacteria is caused by a positively charged atom disengaged from the metal: before it neutralizes the atom becomes negatively charged and causes increased

growth of bacteria, the so-called marginal elevation and double wall. MURTO was the first who described the latter in detail.

Oligodynamic action is very similar to catalysis, which is caused by non-neutral atoms as well. The oligodynamic action is not radioactive, for instance U does not produce any effect. The heavy metals Au and Pt have a weakly checking effect, but their positive charge is still responsible for a small zone round them which is devoid of bacteria. The easily soluble metals Cu, Ag, Zn and Cd are more highly effective. The effect of volatile elements like As, Sb and Hg increases with the size of their surfaces. Even when under the influence of chemical forces the elements retain this property which is also found in salts and compounds and which moreover can not be eliminated by any smelting, coagulation, pressure or vacuum. Oligodynamic action is found in elements with an atomic volume of 8—24. Harmful effect characterises elements with increasing atomic weight and volume. Elements with decreasing atomic volume and increasing atomic weight are stimulating and are originally negatively charged. MURTO is of the opinion that all oligodynamic phenomena are explained by his theory on the migration of atoms and the variation in the charge, viz.: rectilinear course, transmission in water and air, diffusion and sensitiveness to organic colloidal substances. The fact that different metals act differently on various bacteria is considered by MURTO as very important when the pharmacodynamic properties of metals are discussed.

HÄMÄLÄINEN, 1929, has studied, in MURTO's laboratory, the oligodynamic effect of various silver salts on *Staphylococcus aureus*. He used 42 different silver salts and colloidal preparations in petri dishes into which were inoculated cultures of bacteria according to the method suggested by MURTO. Easily soluble salts, from silver nitrate to silver salicylate, produced a comparatively small zone devoid of bacteria. Silver chromate, silver arsenite and especially silver cyanide, all of which are not easily soluble, produced a stronger effect. A marginal elevation, well definable with silver chromate, is not produced by alkalic compounds. HÄMÄLÄINEN explains the formation of the bacteria free region and marginal elevation as follows: the element, Ag, ejects the silver atom with its positive charge and affects harmfully all bacteria in the growth free region; reaching the periphery the charge changes to a negative one, promotes the growth of bacteria and provokes a marginal elevation. The effect of silver salts on *Staphylococcus aureus* is chiefly due to the oligodynamic effect of silver and

not so much to the solubility of the salt. Consequently, HÄMÄLÄINEN is of the same opinion as MURTO.

HORELLI, 1930, has also studied oligodynamics in MURTO's laboratory and has examined the effect of 44 different elements on 23 Mycobacteria, following MURTO's methods. Metals in solid as well as powder form were used. The quantity of bacteria was always the same: approximately $\frac{1}{4}$ of a normal loop. Cultures were kept in optimal temperature and the readings were done when the elements reached their highest effect. MURTO's statement was confirmed in so far that Mycobacteria experienced the strongest preventive influence from As, Sb, Tl; Cd, Hg, Zn and Ge were also powerful in action and a definite effect was further produced by Co, Rd, Ir, Cu, Ag, Bi, In, Te, (Mo).

The following proved stimulating to the growth of Mycobacteria: Di, W, U, Mn, Ni, Er, Y, Fe, Mo. La, Ce, Th, Ta, Al, (Se), V, (Pb), (Sn), Au, B, Ti were indifferent and accidental whereas Mg, C, Si, Zr, Nb, Cr, Pd, (Pt) failed to produce any effect.

A marginal elevation is produced by As, Sb, Cd, Hg, Zn, Co, Rd, Cu, Ag, Bi, Ge, (Mo), and a double one by Sb, Cd, Zn, Co, (Mo). As, Sb, Tl and Hg effected very strongly tubercle bacilli of human as well as bovine types and BCG, cultivated air-tight at 37° on potatoes or glycerine-agar. Then followed Cd, Zn, Ge, Cu, Bi, Co, Ir, Rd, Ag, In. The other metals did not produce any effect. In respect of their reaction to different metals Mycobacteria are a group in themselves, a fact which MURTO had already ascertained when experimenting with two of them.

HORELLI's opinion is that nothing but MURTO's theory on the preventive and promoting effects of different elements is able to explain satisfactorily his own results.

RIED, 1930, radiated by ultraviolet rays liquid fat in which fine Fe-particles were suspended. After filtering and repeated rinsing with ether he enclosed Fe in a cellophane capsule and allowed it to act on a photographic plate at 3 mm's distance and actually obtained a picture of the metal. He checked the result by repeating the test with non-radiated metal and the result proved negative.

RIED is of the opinion that the photographic effect was entirely due to the radiation from the metal. He also tried the effect of radiated Sn and Al on cultures of yeast cells. He dissolved 0.4—1.0 gr. yeast in 300 gr. water of which 30 c.c. were placed in a petri dish and the metal was attached to the lid. Radiated Sn increased the

growth of the culture whereas radiated Al had the opposite effect. Another variation was tried: radiated fat was in the dish, to the lid of which a metal foil was attached during 4 days and then transferred into another dish with yeast cells. This foil exercised a stronger effect than the non-radiated control foil.

According to RIED the increase of the «activating» effect of rays on fat in the presence of metals probably does not depend solely on the catalytic action of metals. The presence of radiated fat alters the photochic or photochemical and biological properties of metals. The photochic property is not constant, neither is the photochemical one which is still as good as unknown. The photochic property of metals and parallel with it their biological and photochemical effects are subject to changes in the temperature. The above mentioned effect on metals is not solely produced by direct exposure to rays, even an indirect action of rays, transferred from radiated substances, fat especially, achieves a similar effect.

RIED has also found in 1931 that metals can be teleactive, due to an emission of unknown quality. The effect of contact between metals and bacteria is not solely due to ions and the influence of the above mentioned emission cannot be denied. Non-radiated metals produce an effect which is dissimilar to that of metals treated with ultra violet rays; whether owing to teleaction or to the effect of direct contact with bacteria cultures. pH also plays a part in this action of metals; radiated metals cause acidity in the medium, non-radiated metals, however, do not. Through the emission from metals as well as by strongly acetous or alkaline media a change is wrought in the expression of the life of bacteria, but after some generations it reverts to the natural state.

The effect of colloidal elements on bacteria.

CERNOVODEANU and HENRI investigated in 1906 the influence of the colloidal metals Ag, Pt, Au, Pd, Cu, Mn and Hg on *Staphylococcus*, *B. typhi* and *B. anthracis*. Ag was effective in a solution of 1:50000, whereby *B. coli* proved to be the more resistant of the two. The effect of these metals increased in accordance with the dispersion phase.

CERNOVODEANU and STODEL found, in 1908, that electrically produced colloidal mercury has greater antiseptic effect than sublimate. In 10 c.c. gelatine a single drop of colloidal Hg = 0.000025 gr.

was sufficient to prevent the development of *Bc. Friedländer*, *B. typhi* and *Staphylococcus*, and 3 drops stopped their growth altogether.

LASAGNA, 1907, found that the solution of colloidal gold in vitro is ineffective against cultures of *B. typhi* and *Staphylococcus pyogenes aureus*.

BIASIOTTI found in 1909 that colloidal silver in a concentration of 1 : 100000 destroys *Staphylococcus (pyogenes) aureus*, *B. typhi* and *Corynebact. diphtheriae* within a very few hours. Undissolved colloidal silver acts on *B. anthracis*, but leaves *Mycobact. tuberculosis* unaffected. After contact with colloidal silver neither *B. anthracis* nor *Staphylococcus* could be stained by GRAM's method, which can only mean that the metal has become chemically incorporated with the bacteria.

FOÀ and AGGAZZOTTI found in 1909 that 1 per cent kalomelol, colloidal bismuth and colloidal ferrous hydrate fail to produce any effect on bacteria. 7 hours are sufficient for hyrgol to destroy *B. typhi*, *Diploc. pneumoniae*, *Staphylococcus*, *B. pestis* and *Vibro cholerae* in 5 per cent cultures. *B. pyocyaneum*, *B. dysenteriae*, *Corynebact. diphtheriae* and *Pneumococcus* are destroyed within 24 hours under similar conditions. The spores of *B. anthracis* are not affected. 0.0125 per cent colloidal Pt and Au destroy *B. typhi* and *Corynebact. diphtheriae* in 24 hours, all other micro-organisms which have been used for experiments remain alive. 0.5 per cent collargol kills all used bacteria in 12 hours. 0.007 per cent electrically produced colloidal silver, olive green and with larger grains, failed to destroy the bacteria in 24 hours but the effect of the brown fine grained variety became deadly within 7 hours. 0.05 per cent colloidal arsenic sulphide kills all bacteria in 7 hours.

BRUNTZ and SPILLMAN demonstrated in 1911 the germicidal properties of colloidal metals in vitro. However, they are of the opinion that the therapeutic properties of these metals are not directly dependent upon the circumstance mentioned, but are rather due to the hyperleukocytosis appearing in the organism after the injection of colloidal metal.

VOIGT, 1914, has shown ultramicroscopically the existence of minute quantities of colloidal silver in tissues. He points out the practical importance of this method in the case of certain infections in order to ascertain whether the micro-organism has absorbed metal. This is of importance also at experimental patho-

logic and pharmacodynamic investigations. In 1925, using the same method, he succeeded in showing that, during oligodynamic experiments in agar with Hg, the metal existed in colloidal form.

RISLER has demonstrated in 1929 that radiated colloidal silver has the power to destroy the most common intestinal microbes, especially *B. coli*, *Proteus*-, Shiga- and Typhoid-bacteria and *Enterococcus*. This a question of a combined physical and chemical effect. The destruction of bacteria can be effectuated by colloidal silver alone, but the process is accelerated by the use of the radiated variety.

The effect of radioactive substances on bacteria.

BECQUEREL studied in 1913 the effect of torium and uranium nitrate on the tubercle bacillus in glycerine-bouillon. He found that the toxic qualities of uranium salts were higher than of thorium salts, inasmuch as the former evoked degenerate forms in the bacillus.

AGULHON and SAZERAC, 1913, demonstrated the toxic effect of uranium nitrate on *B. pyocyaneum* in a solution of 1 : 200 and of uranium acetate in a solution of 1 : 500. Concentrations of 1 : 100 and 1 : 1000 increase the formation of pyocyanine. In concentrations below 1 : 50,000 uranium salts are still harmful in a certain degree. The metal itself has an effect similar to that of the salts.

AGULHON and MILE ROBERT, 1914, studied the effect of colloidal uranium on *B. pyocyaneum* and demonstrated increased growth and formation of pyocyanine when the metal was used in a concentration of 1 : 200000. Colloidal uranium increases the formation of pyocyanine in a strain of bacteria which normally produces itself, but cannot achieve the formation of pyocyanine in a strain which lacks this capacity.

CLUZET, ROCHAIX and KOFMAN, 1920, found that a culture of *B. pyocyaneum* which is 24 hours old escapes the influence of radium. Quite fresh cultures are impeded in their development and get destroyed if their earlier development has been hindered, e.g. by having been kept in a refrigerator. Radium affects bacteria, but not the medium. In order to destroy fresh cultures of *B. pyocyaneum* in pepton-water at 0°, 8400 mg/hours are needed when a platinum tube with 50 mg. $\text{RaBr}_2 \cdot \text{H}_2\text{O}$ is dipped in the culture. The gamma-rays are not active hereat, but the secondary β -rays radiated by platinum. The above mentioned scientists also tried the effect of radium on *B. typhi*. The full force of the bactericidal effect of radium is

noticeable only a few days after being removed from its influence. This effect varies not only with different bacteria, but even with different kinds of the same bacteria. The acting agents are the secondary β -rays.

During their experiment in the biology of uranium STOKLASA and PĚNKAVA, 1928, studied amongst other things the effect of small quantities of uranyl nitrate on bacteria. They tested the capacity of *Azotobacter chroococcum* to bind the nitrogen of the air. They found that it binds considerably more nitrogen with uranyl nitrate, 0,000,003 125—0,001 gramme-atoms per litre, than without. The best effect was obtained with the concentrations 0,000,003 125—0,000 0125 gramme-atoms per litre. The α -rays are the active ones. They further investigated the effect of uranyl nitrate on bacteria which split nitrogenous substances and form ammonia as final product. The bacteria used were *B. mycoides*, *B. mesentericus vulgaris* and *B. megaterium*. The substance was used in different concentrations, with *B. mycoides* 0,000 003 125—0,000 5 gramme-atoms per litre, with the two others the upper limit was 0,001. Uranyl nitrate did not stimulate the ammonization process. They also studied the influence of 0,000 0 125—0,0001 gramme-atoms uranyl nitrate on denitrification processes with *B. Hartlebii* and *B. fluorescens liquefaciens*; these processes were not stimulated, but the growth of new masses of bacteria increased and this result, because of the α -rays, is due to the radioactivity of the element. They point out the vital importance of the fact that dissolved uranium, far from affecting the splitting of HNO_3 into elementary nitrogen, stimulates further growth of organic substances.

MURTO has shown that U has no oligodynamic effect and Po's place in his metal-tables is such that it should be inactive.

CHAPTER 6.

The effect of electrolytes in oligodynamics.

SPIRO, 1916, is of the opinion that the oligodynamic properties of copper run parallel to its solubility. The addition of certain salts increases the solubility of metallic copper, whereas other salts act in the opposite direction. Copper dissolves well in a solution of sodium sulphite, not so in NaCNS-solution. If the former is added to the culture media in a petri dish, the zone which is devoid of bacteria increases considerably, whereas an addition of the latter curtails the oligodynamic effect of copper. The presence of NaCl and NaJ stimulates the oligodynamic effect of copper in vitro; this is also the case with KCl and partly with $MgCl_2$, but not with corresponding sulphates. SPIRO says that there is no antagonism between Cu and Na; the rise or decline of the oligodynamic properties of copper compounds depends on their anions. Cu acts chiefly as cupri-ion. The solubility of the metal increases in the presence of such inorganic salts the anions of which have but little affinity in respect of the metal; this solubility is checked as soon as the formation of such complex compounds takes place which settle on the surface of copper, thereby being an obstacle to the dissolution. The cells contain certain substances which form a compound with the copper and decrease the osmotic counter-pressure of elementary Cu-ions; thus there is a continued ionization of metallic Cu and a dissolution with poisonous effect.

According to EGGERTH, 1924, the stability and potentiality of a suspension of *B. coli* does not depend solely upon the bacteria strain and the suspension medium, but on the reaction of the medium as well. The negative charge of bacteria decreases in acetous medium and with some strains a positive charge can ensue. Changes in stability are accompanied by changes in potentiality.

If bacteria, treated by acid are rinsed with a neutral or mildly alkaline solution, the original potentiality does not become restored.

These changes are caused by two circumstances: 1) a soluble proteine is extracted from the surface of the cell, and 2) another, irreversible change takes place in the cell or its membrane.

LEITNER, 1929, found that an addition of NaCl, in rising concentration to copper activated water, acts as a check on the oligodynamic action. The checking effect increases at first and is followed by a gradual decrease. The top-mark is reached in an average concentration, whereas higher and lower concentrations have but a curtailed effect. This is invariably shown by all tests performed under the same conditions. Other chlorides like K, Ca and Al as well as some potassium salts show the same checking curve. The addition of minute quantities of salt increases the oligodynamic action. An assumption that complex compounds are responsible, according to SPIRO and DOERR, is not a sufficient explanation. A combined physical and chemical process in the liquid outside the bacteria, a reaction between Cu, Na and Cl-ions cannot explain the increase of oligodynamic action through small quantities of salt or the growth impeding effect of electrolytes. Of utmost importance in this case is the series of processes taking place inside or on the surface of bacteria.

In 1930 LEITNER used oligodynamic Cu-water and B.coli in his experiments with different salts. To the first group belong NaCl, KCl, CaCl_2 , MgSO_4 , i.e. the salts of metals from alkalies and alkaline kinds of earth; all these are salts of acids more or less strong. With the addition of these electrolytes the oligodynamic action was found to decline in accordance with the concentration of salt and was at its maximum at $M/4$ — $M/16$. The checking effect was estimated at 50—70 per cent. The quantity of ions in the solution did not diminish. The second group embraces salts of weak acids, viz. potassiumoxalate, potassiumcitrate, ammoniumoxalate, the addition of which resulted in a harmful action due to the formation of complex salts with Cu. The third group consists of salts of very weak acids or hydroxides, viz. potassiumcarbonate, natriumphosphate, natriumacetate, potassiumacetate, they are very weak when in the state of dissociation, react in an alkaline way and contain OH in abundance. Addition of such substances had no checking effect on the bactericidal action.

An investigation proved that the concentration of hydrogen ions affects the bactericidal property of heavy metals; in weak concentrations the acids produce an impeding effect, not the hydrox-

ides. Earlier there was the question of salts belonging to the first group not reducing the number of disengaged Cu-ions in the solution, but there is nevertheless a checking action. The cause must be looked for in the bacteria themselves. There are two ways to explain the process of the harmful action of acids: either a small quantity of metal ions are bound by bacteria, or else the electrolyte endows bacteria with greater resistance to metals.

LEITNER used a highly diluted AgNO_3 solution containing 10^{-5} gr Ag per litre. He found that after 20 minutes 70 per cent of the silver had become absorbed by bacteria. The addition of acetic-acid, 0,1 c.c. n/100 to 100 c.c. AgNO_3 solution, bringing the concentration of acid to n/1000 and leaving the concentration of silver unaltered, resulted in only 24 per cent of the silver becoming absorbed by bacteria. This is due to the loss in the negative electrostatic charge of bacteria caused by the addition of acid. Bacteria in suspension are negatively charged and are therefore attracting positive ions. This negative charge is not constant, but depends on the electrolytes of the suspension, as already shown by NORTHROP and DE KRUIF. NaCl in rising concentration weakens the negative charge. Typhoid-bacteria with an n/10000 NaCl-solution have a charge of 30 millivolts and with an n/10 solution, 2 millivolts. With an addition of NaCl, CaCl_2 , MgSO_4 in rising molecular concentration the checking effect shows a corresponding increase, the charge in bacteria grows weaker and a minor quantity of ions becomes absorbed by bacteria. An addition of OH increases the charge in bacteria; an increased absorption takes place at the same time and the bactericidal effect of Cu undergoes an increase as well.

GOTTSCHALK, 1932, has studied the influence of different circumstances on the oligodynamic properties of silver. He used *B. coli*, 800—2400 to the c.c. If the number of bacteria is increased, they are quickly destroyed to but a small group, but these few remaining ones can show great resistance and even after 8 hours it was possible to come across live bacteria. The number of bacteria is not as important as is the volume of water containing bacteria to the given metal unit. A 32-fold dilution causes a somewhat stronger preventive effect than a 1000-fold increase in the number of bacteria. When an oligodynamic metal is active in a solution containing an electrolyte, it depends on the nature of the latter whether the oligodynamic action is promoted or prevented in comparison with its effect in distilled water. The influence of electrolytes on the mani-

festation of oligodynamic forces is possible in two ways: the solubility of the metal in electrolyte water can be changed, or else the effect of the dissolved metal on bacteria can be altered by electrolytes. In the foregoing it was shown by LEITNER that this electrolytic effect is due to the changed absorptive capacity of bacteria towards metal ions.

According to GOTTSCHALK there are numerous electrolytes in varying concentrations dissolved in spring-water. He tested the water from 3 different springs having different electrolytic capacities and found that of 2400 bacteria in the c.c. after 2 hours none were left in the water with the weakest electrolytic concentration. In slightly stronger electrolytic water 8 colonies were left after a similar period of time, whereas the strongest electrolytic water had 50 colonies in it after 2 hours and 6 colonies after a further 3 hours; in 4 hours all liquids proved to be sterile and a test with distilled water produced sterility in 2 hours.

CHAPTER 7.

The effect of different elements in connection with their place in the periodic system.

Some scientists have studied the germicidal properties of various elements in connection with their classification in the periodic system or according to their chemical valency.

LUSINI, 1910, is under the impression, that even if the bactericidal capacity of cations does not stand in direct proportion to the atomic weight, there do exist certain relations according to which the heaviest cations generally exercise the greatest influence. Alkaline metals generally have a weaker action than metals of alkaline kinds of earth. The disinfectant qualities of the former are in proportion to their atomic weight, whilst with the latter the proportion is reversed. Elements of the magnesia group are characterised by a greater activity than those of the two foregoing groups, usually, the heavier the atom, the stronger the action. This is also the case with the elements in the mercury group. The elements of the Fe-group have but weak disinfectant properties, but here too the power of action is governed by the weight of atoms. The tin-lead group is more effective and again the action stands in proportion to the atomic weight.

According to MESSERSCHMIDT, 1916, some elements in certain groups of the periodic system act stronger than others, but elements which possess germicidal properties are spread over the whole system. Most effective are Cu, Sb, As, Mg and Zn; less pronounced is the action of Ni, Ag, Bi and Cd; to a certain extent also of Pb.

FRIEDENTHAL, 1919, is of the opinion that if an element forms a part of ions with different electric charge, its effect is likewise apt to vary. The effect of substances *in vitro* cannot be compared with the effect *in vivo*, because certain oxidating and reducing processes take place in the organism. Of all the elements in group I, Ag has the greatest effect, even when in colloidal form. Amongst the a-metals of group II, Ba is the most effective, although much weaker

than Ag. The b-element Cd is similar in effect to Ag, whereas the action of Hg is considerably stronger. In group III compounds of B and Al are more powerful than the a-elements in group II; Tl is also very effective. In group IV Pb is active and C, as an element, inactive. In this group the effect increases with augmented atomic weight, whereas group V is an illustration as to the contrarity. In group VI the b-element O is the most powerful of all. Group VII shows increased effect of b-elements in accordance with the increase of atomic weight. In group VIII Fe, Co, Ni, Ru, Rh, Pd, Os, Ir and Pt are inactive even in colloidal form, but Fe and Os are influential as ions.

MURTO's investigations are described in chapter 6.

According to WINSLOW and DOLLOFF, 1928, the growth of bacteria is increased by metallic cations as Na, K, Ca, Mg, Pb and Hg in low concentration, but higher concentrations achieve a preventive effect. Na, K and Mg have the same degree of effect on *B. coli*, but from the point of view of quantity Mg is 8 times as powerful as ions with one valency. Hg- and H-ions have a thousand times the power of alkalies.

WINSLOW and HAYWOOD, 1931, examined Na, K, Li, Ba, Mg, Ca, Mn, Zn and Cd in respect of their effect on *B. coli*. All cations in weak concentrations have the same effect in common, viz: a stimulating action in respect of bacteria growth. Each of the above mentioned metals has a certain individual power in which the growth of bacteria is impeded. Assuming Na = 1, the power of the other cations may be indicated as follows: K 1,2; Li 3,4; Ba 5,0; Mg 9,4; Ca 12,0; Mn 400; Zn 700; Cd 3000.

FISCHL, 1931, says when referring to the relation between the periodic system of elements and their chemotherapeutic action, that out of 12 elements which present chemotherapeutics consider as active, 4 belong to group V of the system: V, As, Sb and Bi; 3 belong to group I: Cu, Ag and Au. Amongst the horizontal lines the VI:th shows four elements: Ag, Sb, Te and J; the VIII:th four: Au, Hg, Pt and Bi; the IV:th three: Cu, Ga and As. It is of interest to note the connection between the chemotherapeutic effect and the physical characteristics. Chemotherapeutically active elements are generally metalloids of metallic appearance and heavy metals. Most of the 12 active elements, viz. V, Ag, Sb, Te, J, Hg and Bi in elementary form are active as powder and colloids, whereas Ga, As, Au and Pt are absolutely inactive in this form, although their compounds may

show good results. Concerning elements of a similar group one cannot say anything definite in respect of law-bound relations between chemical constitution and effect, still less when speaking summarily of all the elements. Further investigations and continued study of chemotherapeutics apparently belong to the future. There is possibly an empiric course to find new active substances.

BRUNNER has experimented with Sb and its effect on dogs and rats and in 1912 he came to the conclusion that one can distinguish between weak and strongly active Sb-compounds. The difference lies in the circumstance, that the element is bound in the molecule in different ways. Sb has three valencies in strong preparations and five valencies in the weak ones.

FRIEDBERGER and JOACHIMOGLU, 1917, used As and Sb with three and five valencies whilst experimenting with *B. prodigiosum*, *Staphylococcus*, *B. typhi*, «*Vibrio Stade*», *Vibrio Metschnikoff* and a Nagana-strain. They found that As with three valencies affected all microorganisms quicker than with five valencies and Sb with three valencies was quicker than with five valencies in affecting the Nagana-strain.

CHAPTER 8.

Activation of glass vessels in oligodynamic experiments.

According to HEYDWEILLER and KOPFERMANN, 1910, it is possible for many metals to enter glass by diffusion, or rather electrolytically. The most favourable conditions for it are found in temperatures exceeding 200°C . With Ag and Cu this process is noticeable, for instance, by the glass changing its colour owing to the distribution of metal.

E. WARBURG has shown in 1910 that only positive ions, metal ions, have the capacity to enter glass during the process of diffusion and electrolysis.

GÜNTHER SCHULZ, 1913, found that in a temperature of 250°C Ag diffuses out of AgNO_3 into glass, this process taking place by way of ions. Each entering silver ion causes a sodium ion to emerge. The influencing quantity of Ag is in proportion to the concentration of ions on the surface of the glass.

V. NÄGELI already was of the opinion that the so-called activation of glass vessels in oligodynamic tests is due to the glass taking up metal particles from the solution and returning them again to the liquid when refilled with fresh water. This opinion is shared by others, e.g. SCHLOSSBERGER, FALTA and RICHTER-QUITTNER, STRECK, SÜPFLE and EICHBAUM. V. LINDEN had it analysed how much Cu could be absorbed by 1 gr. glass powder out of 1 100 mol. solution of CuSO_4 . The result was: 0.0385 gr. after 1 hour and 0.0490 gr. after 8 hours. The metal is taken up partly by absorption and partly a copper silicate is formed which is difficult to dissolve. In the former case the absorbed metal is so effectively bound by the glass that it is not returned to fresh supplies of water. Thus glass can bind considerable quantities of Cu. Water containing bacteria may separate more Cu from glass than such, devoid of bacteria. This is

due to the immense surface of bacteria which offers better possibilities for absorption than the walls of the glass.

In 1928 FREUNDLICH and SÖLLNER showed that when pure Ag, 40 cm² to 1000 c.c. water, is allowed to act during 72 hours, Ag is found in the water in a quantity of 2×10^{-5} gr. per litre. If algae, Spirogyra, are added to this solution, they absorb the silver and undergo the changes mentioned by v. NÄGELI. The algae absorb 79 per cent of the silver contained in the water. There was 5×10^{-5} gr. silver to a gramme dry substance algae. This characterizes the action of silver as the effect of Ag as element. FREUNDLICH and SÖLLNER have thus shown the existence of silver both in water and in the organisms. Ag is contained in water in the form of an ion. Similar changes in algae can be wrought by an AgNO₃ solution containing Ag in the same concentration. In all probability the dissolution of metallic Ag takes place under the influence of oxygen and carbon dioxide. The circumstance, that glass vessels, which have been in touch for a time with a silver containing liquid, still retain their oligodynamic properties even after having been freshly filled, is due to the absorption of silver ions by the glass owing to its permutit structure which allows them to penetrate. With the addition of fresh liquid the silver ions can dissolve again and be taken up by silver absorbing cells which take the silver off the glass walls. The absorption of silver by glass and its continued penetration into the latter is connected with the fact that Ag cannot be traced when a dissolved AgNO₃ solution is vaporized in a glass vessel; this can be achieved, however, by the use of a quartz vessel. Quantity tests have shown that when one tried to disengage Ag again by using diluted HNO₃ in a glass vessel submitted to the vaporization of AgNO₃, about 3 per cent only could be reclaimed, whereas fully 64 per cent of the original quantity could be released by using quartz vessels.

WRIGHT found, in 1928, that glass powder absorbs the Ag-ions of a water solution of AgNO₃.

The activation of glass vessels and subsequent activation of freshly filled water, as shown by a number of scientists, is due to the diffusion and exchange of ions in the glass, which may be considered as a permutit (SMITH-D'ANS). According to FREUNDLICH a permutit is able to exchange its own alkali metal, in respect of quantity, to another metal, whereby the qualities of the permutit are retained for the most part.

CHAPTER 9.

Discoloration of bacteria under the influence of physicochemical agents.

WIRGIN found, in 1902, that *B. prodigiosum* and *B. pyocyaneum*, which under ordinary conditions show a substantial formation of pigment, lose this property as soon as alcohol is added to the culture media. At usual room-temperature *B. prodigiosum* was still forming pigment when in 5 per cent alcoholic agar, but in agar with a higher percentage the formation of pigment ceased, although bacteria were growing freely otherwise. When bacteria were allowed to grow from a fortnight to a few months on agar of the above description, without pigment, and new cultures were sawn out from these colourless ones on ordinary agar, it was observed that the bacteria recovered their colour to the full extent. Some scientists have produced temporarily colourless cultures, but only CHARRIN and PHISALIX, 1892, succeeded in producing bacteria constantly free from pigment; they used *B. pyocyaneum* in high temperature, 42.5° C. The fourth generation proved already colourless in cultures in serum, agar and bouillon. The colour was restored by passage through a rabbit. After 5 generations, in 42.5° C, the pigment could not be restored any more.

FRIEDMANN found in 1931 that *B. pyocyaneum* loses its pigment when completely shut off from the air. Admission of air restores the pigment again. In the absence of oxygen bacteria reduce the pigment to a colourless leuco base which by oxygen is reoxidized into a dye. Pyocyanine reacts as an autooxidable acceptor of labile hydrogen in the bacteria. Pyocyanine stimulates the breathing of bacteria. Without pyocyanine bacteria use a certain amount of oxygen. In the presence of pyocyanine breathing increases up to 100 per cent. Pyocyanine affects the actual breathing of bacteria, not the accessory one. Pyocyanine catalyzes a certain substance which is intimately connected with the bacteria, probably bacteria lipoids and poly-

saccarides, or certain products of theirs. The fact that the bacteria have an evident metabolism even when pyocyanine is absent, leads one to suppose that the latter is not of primary importance in the process of breathing. Experiments show that pyocyanine is but an accessory ferment the effect of which is dependent on the presence of one or more breathing ferments; cytochrome is probably amongst the latter. The effect of pyocyanine stands in connection with a ferment sensitive to KCN and CO. Accelerated breathing, caused by pyocyanine, does not concern *B. pyocyaneum* alone, even other bacteria, containing cytochrome, are stimulated in their functions; e.g. *Staphylococcus*, *Pneumococcus* and red blood corpuscles from rabbit. The affinity of pyocyanine to proteins is weak, but it is strong to lipoids. The breathing of cells can be increased up to a 24-fold intensity.

LAGRANGE has shown in 1932 that when coli bacteria on endo-agar are covered up with lead foil placed 5 to 10 mm. above the culture, the colonies lose their dye. Ag, Fe and Ni are also effective. Brass and Cu are inactive, so is glimmer. Colourless coli cultures affect in their turn such cultures as are still in possession of their dye. This effect is weakened to a certain extent by Cu, brass- and glimmer-foil, also by Al and glass. The metal foils become highly oxidized, particularly lead foils.

CHAPTER 10.

The author's experiments.

The number of micro-organisms used for the tests is 40. They are obtained from laboratory cultures which were inoculated anew in order to obtain new cultures whereupon they were taken into use. The bacteria were not controlled in regard to species as at these tests the chief intention was to determine the influence of different elements and the bacteria are to be considered more as indicators of the effect of the substances.

For the final test series only such b-elements from the periodic system were used as had shown definite injurious effect on micro-organisms in previous tests of other investigators. No such elements were used which would macroscopically greatly alter the culture media and either dissolve therein or cause it to coagulate, e.g. the b-elements Zn and Tl. The former dissolves visibly in the culture media within 24 hours and coagulates it. Tl dissolves within a few seconds and can be easily proved to exist therein. A few experiments were also made with a-elements. Heretofore no similar oligodynamic experiments were ever made with Be, but it proved now to be absolutely inactive. Al is also passive, apparently due to the protective layer of oxide round it which prevents it from exercising any effect. Mo was used only to demonstrate the spreading of it in the culture media. The elements were used in solid form, except Te, Co and Mo which were adopted in the form of powder, whenever occasion arose, to show their effect with all possible distinctness.

The following elements of group I of the periodic system were used: Cu Ag and Au. Group II was represented by Cd and Hg. Zn was out of the question owing to reasons already mentioned above. Elements belonging to the groups III and IV were not used, because previous tests had shown already that, e.g., Sn and Pb have but little effect and Tl dissolves swiftly in the culture media. From group V were chosen As, Sb and Bi which are well adapted for such

tests. Te alone was selected from group VI, as Se had proved itself inactive. Group VII was left out entirely. In group VIII Co alone proved oligodynamic, a few experiments with Fe, Ni, Pd and Pt showed them to be inactive. The total number of elements was 10, viz.: Cu, Ag, Au, Cd, Hg, As, Sb, Bi, Te and Co.

The technic of the tests was as follows: 15 c.c agar was melted and poured into petri dishes with a radius of 10 cm: as soon as the surface of the culture media became completely dry, avoiding syneresis, inoculation took place. In all tests half a standard loopfull bacteria was used, taken from fresh pure cultures. The dish was placed on a rotating table and the bacteria were spread out with a wide platinum triangle. The elements were all thoroughly cleaned and between the tests they were kept in separate, well closed glass containers. Sterile pincers were used to place the elements on the agar at a suitable distance from each other. Very strongly active elements were used separately. Dishes prepared in this way were kept in temperature suitable for every micro-organism. The culture was allowed to develop and daily proofs were taken from the region devoid of bacteria and transplanted into other dishes. The results were registered on the following day or later, depending on the time taken by the bacteria to develop. In a few cases no bacteria free region had formed round the element and the sample had to be taken from a place immediately under the element. In the following bacteria free zone or bacteria free region shall denote the place in the agar which is nearest the element and where bacteria growth is not detected by the naked eye. Every micro-organism was passed through three series of tests of 14 days each and the average result was registered. Certain bacteria had to pass through a greater number of tests, all according to prevailing circumstances. These tests took a considerable time because the bacteria lived long before succumbing to the influence of the elements. These were cleansed daily in order to avoid the formation of a protecting layer of agar colloid which would prevent them from exercising their influence unhampered. Hg was removed daily with the aid of a pipette and replaced by a similar drop of sterile Hg. In order to preserve the agar humid the dishes were kept in closed glass containers with damp cotton or filter paper at the bottom. This prevented the culture media from drying up. Parallel testing proofs were always at the same time taken from such places in the dishes where normal growth of bacteria took place.

In the following, the influence of the different elements on each bacteria is given, whereby the figure standing after the symbol of the element denotes, in mm., the bacteria free zone. The appearance of the border line limiting the region where bacteria growth is still evident is described as either sharp or diffuse and it is noted down whether a marginal elevation has been observed or not. Further data cover changes produced by certain elements in either the culture media or the culture, especially by Cu, Te and Co. Such changes do not take place at once, but only after a few days.

A separate series of tables shows how long time different bacteria were able to live under the influence of the elements: »+ + +» denotes normal growth, »+ +» slightly weakened growth, »+» considerably weakened growth and »—» absence of new colonies. In the text bacteria shall be denoted by Roman figures.

Micro-organisms used in the experiments.

- I: *Sarcina flava*.
- II: *Micrococcus candicans*.
- III: *Micrococcus (pyogenes) aureus*.
- IV: *Micrococcus (pyogenes) citreus*.
- V: *Micrococcus (pyogenes) albus*.
- VI: *Micrococcus Freudenreichii*.
- VII: *Bacillus abortus* (typ. human. Bang).
- VIII: *Bacillus abortus* (typ. bovin.).
- IX: *Bacterium (lactis) viscosum*.
- X: *Bacterium (lactis) amarum*.
- XI: *Bacterium typhi*.
- XII: *Bacterium (faecalis) alcaligenes*.
- XIII: *Bacterium paratyphi B*.
- XIV: *Bacterium (typhi) murium*.
- XV: *Bacterium ratti*.
- XVI: *Bacterium (septicaemiae) murium*.
- XVII: *Bacterium coli*.
- XVIII: *Bacterium coli*, home cultivated strain.
- XIX: *Bacterium prodigiosum*.
- XX: *Pseudomonas caerulea*.
- XXI: *Pseudomonas pyocyanea*.
- XXII: *Pseudomonas putida*.
- XXIII: *Bacterium vulgare*. (*Proteus vulgaris*).

- XXIV: *Bacterium vulgare* X₁₉.
 XXV: *Bacillus mycoides*.
 XXVI: *Bacillus subtilis*.
 XXVII: *Bacillus vulgatus*.
 XXVIII: *Bacillus mesentericus*.
 XXIX: *Bacillus teres*.
 XXX: *Bacillus asterosporus*.
 XXXI: *Vibrio cholerae*.
 XXXII: *Vibrio cholerae* (El Tor).
 XXXIII: *Vibrio proteus* (Finkler-Priori).
 XXXIV: *Vibrio albensis*.
 XXXV: *Vibrio aquatilis* (Rudolfs-Hospital).
 XXXVI: *Corynebacterium pseudodiphtheriticum*.
 XXXVII: *Mycobacterium (phlei) flavum*. (Cultivated by Murto).
 XXXVIII: *Mycobacterium (phlei) rubrum*. (Cultivated by Murto).
 XXXIX: *Actinomyces chromogenes*.
 XL: *Leptothrix*. Sp. i.

I. Sarcina flava.

- Cu: 3 mm, sharp borderline, weak marginal elevation 1 mm. After 1 day a light green margin, 1 mm. wide, outside which the agar has a violet tint; after 4 days the violet colour is 7 mm, after 6 days the light green border is 2 mm. and the violet tint extends 12 mm. from the metal.
- Ag: 1.5 mm, sharp borderline, marginal elevation 1 mm.
- Au: 2 » sharp borderline, after 1 day 1 mm. marginal elevation, the colour of which changes to light brown after 3 days.
- Cd: 10 » sharp borderline.
- Hg: 4 » diffuse borderline.
- As: 8 » » »
- Sb: 12 » sharp »
- Bi: 2 » » » outside it a 6 mm. zone with weak growth and 1 mm. marginal elevation.
- Te: 6 » diffuse borderline, brown coloured colonies 3 mm., after 3 days 7 mm.
- Co: 5 » sharp borderline with 2 mm. marginal elevation. After 2 days 6 mm. wide reddish tint spreads around the metal, after 6 days the red colour extends 12 mm. from the metal.

II. Micrococcus candicans.

Cu: 1.5 mm, sharp borderline.

Ag: 1 » » »

Au: 1 » » »

Cd: 3 » » »

Hg: 8 » diffuse borderline.

As: 9 » » »

Sb: 6 » » »

Bi: 1 » sharp »

Te: 8 » » » outside it 4 mm. of weak growth,
followed by normal growth; after 4 days the colonies
are coloured light brown.

Co: 2 » sharp borderline. After 2 days 4 mm. of red colour
around the metal is visible simultaneously with a
marginal elevation, after 7 days the red colour extends
7 mm.

III. Micrococcus (pyogenes) aureus.

Cu: 4 mm, sharp borderline. After 3 days 2 mm. of violet tint in the
agar around the metal.

Ag: 4 » sharp borderline. After 2 days, marginal elevation 2 mm.

Au: 2 » » » marginal elevation 2 mm.

Cd: 6 » » » » » » »

Hg: 4 » » » After 2 days, marginal elevation 2 mm.

As: 15 » diffuse »

Sb: 8 » sharp »

Bi: 1 » » »

Te: 5 » » » After 2 days the colonies are brown
3 mm.

Co: 4 » sharp borderline, marginal elevation 3 mm. After
3 days 3 mm. of red colour spreads around the metal,
after 6 days 8 mm.

IV. Micrococcus (pyogenes) citreus.

Cu: 3 mm, sharp borderline.

Ag: 3 » » »

Au: 1 » » »

Cd: 7 » » » marginal elevation 2 mm.

Hg: 4 » diffuse »

As: 9 mm, diffuse borderline.
 Sb: 10 » » »
 Bi: 1 » sharp »
 Te: 5 » » » marginal elevation 2 mm.
 Co: 6 » » » » » 0.5 mm.

V. Micrococcus (pyogenes) albus.

Cu: 2 mm, sharp borderline.
 Ag: 1 » » »
 Au: 1 » » »
 Cd: 5 » » » 3 mm. of weak growth, then follows
 a marginal elevation of 2 mm.
 Hg: 2 » sharp borderline.
 As: 7 » diffuse »
 Sb: 6 » » »
 Bi: 2 » sharp »
 Te: 6 » » » After 2 days a 3 mm. wide brown
 coloured zone is visible in the colonies.
 Co: 4 » diffuse borderline.

VI. Micrococcus Freudenreichii.

Cu: 2 mm, sharp borderline.
 Ag: 3 » » » After 6 days 2 mm. of the agar around
 the metal are brown coloured and after 11 days 4 mm.
 Au: 1.5 » sharp borderline, weak marginal elevation.
 Cd: 6 » diffuse »
 Hg: 9 » » »
 As: 11 » » »
 Sb: 11 » sharp » marginal elevation 0.5 mm.
 Bi: 2 » » »
 Te: 5 » » » After 4 days 4 mm. of the colonies are
 brown coloured, after 6 days 8 mm.
 Co: 1.5 » sharp borderline. After 4 days 7 mm. of red colour
 outside the metal, after 8 days 11 mm.

VII. Bacillus abortus. (typ. human. Bang).

Cu: 4 mm, sharp borderline, marginal elevation 2 mm, which is
 brown coloured after 4 days.

- Ag: 2 mm, sharp borderline. After 4 days a 3 mm. wide brown coloured area around the metal.
- Au: 1 » sharp borderline, marginal elevation 0.5 mm.
- Cd: 2 » » » » 1 mm, from the element outwards 4 mm. metallic lustre.
- Hg: 3 » sharp borderline.
- As: 12 » diffuse » 2 mm. of increased growth.
- Sb: 7 » sharp » marginal elevation 1 mm.
- Bi: 1 » » »
- Te: 12 » » marginal elevation 1 mm. After 4 days 3 mm. of the colonies are brown coloured.
- Co: 3 » sharp borderline, marginal elevation 1 mm, outside the metal 4 mm. of red colour, after 6 days 7 mm.

VIII. Bacillus abortus. (typ bovin).

- Cu: 4 mm, sharp borderline. After 4 days 3 mm. of violet colour outside the metal.
- Ag: 5 » » borderline
- Au: 3 » » »
- Cd: 5 » » »
- Hg: 5 » » marginal elevation 0.5 mm.
- As: 9 » diffuse »
- Sb: 7 » sharp »
- Bi: 2 » » »
- Te: 11 » » » 1 mm. brown coloured marginal elevation.
- Co: 4 » sharp » marginal elevation 1 mm. After 4 days 5 mm. of red colour outside the metal.

IX. Bacterium (lactis) viscosum.

- Cu: 2 mm, sharp borderline.
- Ag: 4 » » »
- Au: 3 » » » marginal elevation 0.5 mm.
- Cd: 8 » diffuse »
- Hg: 5 » sharp » after 4 days marginal elevation 2 mm.
- As: 12 » diffuse »
- Sb: 11 » sharp »
- Bi: 0.5 » » »

Te: 2 mm, sharp borderline. After 4 days 6 mm. of the colonies are brown coloured, after 7 days 8 mm.

Co: 2 » sharp borderline, marginal elevation 1 mm. After 4 days 4 mm. of red colour outside the metal, after 8 days 8 mm.

X. Bacterium (lactis) amarum.

Cu: 0.5 mm, sharp borderline.

Ag: 1 » » » after 2 days marginal elevation 2 mm.

Au: 2 » » » , marginal elevation 1 mm.

Cd: 5 » diffuse »

Hg: 3 » sharp » , marginal elevation 1 mm.

As: 7 » diffuse »

Sb: 5 » » »

Bi: 1 » sharp »

Te: 8 » » » , marginal elevation 1 mm. After 4 days 4 mm. of the colonies are brown coloured.

Co: 3 » sharp borderline. After 4 days 3 mm. of red colour outside the metal, after 8 days 6 mm.

XI. Bacterium typhi.

Cu: 1.5 mm, sharp borderline. After 4 days 4 mm. of light violet colour around the metal, after 8 days 7 mm.

Ag: 2 » sharp borderline. After 4 days 3 mm. of brown colour around the metal

Au: 1.5 mm, sharp borderline.

Cd: 3 » » »

Hg: 10 » » »

As: 17 » diffuse »

Sb: 12 » » »

Bi: 1.5 » sharp borderline.

Te: 15 » diffuse » . After 4 days 5 mm. of the colonies are brown coloured, after 6 days 11 mm, after 8 days 15 mm.

Co: 4 » sharp borderline, marginal elevation 0.5 mm. After 4 days 6 mm. of red colour around the metal, after 8 days 9 mm.

XII. Bacterium (faecalis) alcaligenes.

Cu: — the colonies take root in the metal. After 4 days 4 mm. of brown colour around the metal, after 6 days 2 mm. light

green margin is visible around the metal, the brown colour extends 7 mm. from the metal outwards.

Ag: 1 mm, sharp borderline. After 6 days 4 mm. of brown colour around the metal.

Au: 0.5 » sharp borderline.

Cd: 3 » » »

Hg: 2 » » » , marginal elevation 1 mm.

As: 6 » diffuse »

Sb: 4 » » »

Bi: the colonies grow to the metal.

Te: 9 mm, sharp borderline, marginal elevation 2 mm. After 3 days 3 mm. of the colonies are brown.

Co: 2 » sharp borderline, marginal elevation 2 mm. After 4 days 5 mm. of red colour around the metal.

XIII. Bacterium paratyphi B.

Cu: 1 mm, sharp borderline. After 6 days 4 mm. of violet colour around the metal.

Ag: 1.5 » sharp borderline. After 6 days 3 mm. of brown colour around the metal.

Au: 2 » sharp borderline, marginal elevation 0.5 mm.

Cd: 2 » » »

Hg: 10 » » »

As: 16 » diffuse borderline.

Sb: 11 » sharp »

Bi: 1.5 » » »

Te: 13 » » » , weak marginal elevation 2 mm. After 4 days 9 mm. of the colonies are brown coloured, after 8 days 16 mm.

Co: 3 » sharp borderline. After 4 days 8 mm. of red colour around the metal.

XIV. Bacterium (typhi) murium.

Cu: 0.5 mm, sharp borderline.

Ag: 0.5 » » »

Au: 1 » » »

Cd: 6 » diffuse »

Hg: 3 » sharp »

As: 13 » diffuse »

Sb: 8 mm, diffuse borderline.
 Bi: 1 » sharp »
 Te: 3 » » » then follows a 3 mm. zone of weak growth and outside it 2 mm. of increased growth.
 Co: 3 » sharp borderline. After 4 days 4 mm. of red colour around the metal.

XV. *Bacterium ratti*.-

Cu: 0.5 mm, sharp borderline.
 Ag: 1 » » »
 Au: 3 » » » , after 3 days marginal elevation 2 mm.
 Cd: 4 » diffuse »
 Hg: 3 » sharp »
 As: 16 » diffuse »
 Sb: 10 » » »
 Bi: 1.5 » sharp »
 Te: 6 » » » . Outside it a 5 mm. zone of weak growth, then follows 2 mm. of increased growth.
 Co: 2 » » » After 6 days 5 mm. of red colour around the metal.

XVI. *Bacterium (septicaemiae) murium*.

Cu: 1 mm, sharp borderline. After 4 days a 1 mm. light green margin encircled by 4 mm. of violet colour.
 Ag: 1 » sharp borderline.
 Au: 3 » » » , marginal elevation 1 mm.
 Cd: 4 » diffuse »
 Hg: 2 » sharp » , marginal elevation 1 mm.
 As: 15 » diffuse »
 Sb: 8 » » »
 Bi: 0.5 » sharp »
 Te: 10 » » » , marginal elevation 2 mm.
 Co: 3 » » » , a 3 mm. zone with weak growth outside it, followed by normal growth. After 4 days 8 mm. of red colour around the metal.

XVII. *Bacterium coli*.

Cu: 3 mm, sharp borderline. After 4 days 3 mm. of violet colour around the metal, after 6 days 2 mm. light green margin, outside which 6 mm. of violet colour.

- Ag: 2 mm, sharp borderline, marginal elevation 1 mm. After 6 days 3 mm. of brown colour around the metal.
- Au: 1 » sharp borderline, marginal elevation 1 mm.
- Cd: 3 » » »
- Hg: 3 » » » , 1 mm. marginal elevation, which grows in 3 days to 3 mm.
- As: 7 » sharp borderline, 1 mm. marginal elevation which grows in 4 days to 2 mm.
- Sb: 5 » sharp borderline, marginal elevation 1 mm.
- Bi: 1 » » »
- Te: 7 » » » , marginal elevation 2 mm, 7 mm. of the colonies are brown, after 4 days 10 mm.
- Co: 3 » sharp borderline, marginal elevation 1 mm.

XVIII. Bacterium coli, home cultivated strain.

- Cu: 2 mm, sharp borderline. After 4 days 2 mm. of violet colour around the metal, after 6 days 2 mm. light green margin, from which the violet zone extends 7 mm. outwards.
- Ag: 1 » sharp borderline, marginal elevation 1 mm.
- Au: 0.5 » » »
- Cd: 2 » » »
- Hg: 3 » » » , marginal elevation 1 mm.
- As: 5 » » » » » 2 »
- Sb: 6 » diffuse »
- Bi: 1 » sharp »
- Te: 4 » » » , marginal elevation 1 mm. After 4 days 5 mm. of the colonies are brown coloured, after 8 days 9 mm.
- Co: 3 » sharp borderline, marginal elevation 1 mm. After 4 days 5 mm. of red colour around the metal.

XIX. Bacterium prodigiosum.

- Cu: 8 mm, diffuse borderline, colonies are grey. After 1 day 1 mm. light green margin around the metal, encircled by 5 mm. of violet colour, after 6 days the light green margin is 2 mm. wide, and the violet colour extends 9 mm. from the metal.
- Ag: 2 » sharp borderline, 1 mm. marginal elevation which grows to 2 mm. in 4 days and becomes light brown; other colonies are grey.

- Au: 1 mm, sharp borderline, 0.5 mm. marginal elevation which becomes brown after 6 days.
- Cd: 2 » sharp borderline, colonies are grey.
- Hg: 6 » » » » »
- As: 10 » diffuse » » »
- Sb: 7 » » » » »
- Bi: 1 » sharp » , marginal elevation 0.5 mm.
- Te: 10 » » » , outside it there is a 3 mm. zone of red colonies, followed by grey ones. After 6 days the colonies are red to the extent of 10 mm. from the borderline outwards.
- Co: 2 » sharp borderline, marginal elevation 2 mm, the colonies are grey.
- B. prodigiosum loses its red colour under the influence of elements. Only with Te the colour remains in the area nearest the metal, the red colonies are encircled by grey colonies. The red colour in the culture extends slowly and is very intensive. The control-colonies are pigmented during the whole time.

XX. *Pseudomonas caerulea*.

- Cu: 1 mm, sharp borderline.
- Ag: 3 » » »
- Au: 1 » » »
- Cd: 7 » diffuse »
- Hg: 5 » sharp »
- As: 16 » diffuse »
- Sb: 12 » sharp borderline, 2 mm. marginal elevation, which grows to 3 mm. in 6 days.
- Bi: 1 » sharp borderline.
- Te: 6 » » »
- Co: 2 » » » , After 4 days 5 mm. of red colour around the metal, after 6 days 8 mm.

XXI. *Pseudomonas pyocyanea*.

- Cu: 1.5 mm, sharp borderline with slightly increased growth, violet colour around the metal. After 4 days the colour extends 8 mm. After 2 days 1 mm. light green margin is visible around the metal, after 6 days the margin is 2 mm. wide and the violet colour is darker.

- Ag: 3 mm, sharp borderline, marginal elevation 1 mm. After 6 days 3 mm. of brown colour around the metal.
- Au: 0.5 » sharp borderline.
- Cd: 1 » » »
- Hg: 4 » » » , marginal elevation 0.5 mm, the bacteria, free zone is blue.
- As: 15 » sharp borderline, the culture shows a 5 mm. grey area in its border, the colour of the agar changes gradually to blue; in bacteria free zone the agar retains its usual colour.
- Sb: 13 » sharp borderline, at the border of the culture there is a 3 mm. grey zone, the colour of which does not change, the agar itself becomes blue except in the bacteria free area, where it retains its ordinary tint.
- Bi: 1 » sharp borderline, marginal elevation 1 mm.
- Te: 8 » diffuse » , 5 mm. of the colonies are brown, the agar in the bacteria free zone is of ordinary appearance.
- Co: 3 » sharp borderline, marginal elevation 2 mm. After 4 days 7 mm. of red tint around the metal, after 8 days 12 mm.

XXII. *Pseudomonas putida*.

- Cu: 0.5 mm, sharp borderline. After 2 days a 1 mm. light green rim around the metal, encircled by 4 mm. of violet colour, after 6 days the latter grows to 8 mm.
- Ag: 1 » sharp borderline, marginal elevation 0.5 mm. After 6 days 3 mm. of brown colour around the metal.
- Au: 1 » sharp borderline.
- Cd: 5 » diffuse »
- Hg: 3 » sharp »
- As: 2 » diffuse »
- Sb: 6 » » »
- Bi: 1 » sharp »
- Te: 2 » » » , 4 mm. of brown colour in the colonies.
- Co: 3 » » » . After 4 days 5 mm. of red colour around the metal.

XXIII. *Bacterium vulgare*. (*Proteus vulgaris*).

- Cu: 2 mm, sharp borderline. After 4 days 1 mm. light green rim around the metal and 4 mm. of violet colour, after 8 days the latter is 6 mm. wide.

Ag:	3 mm,	sharp	borderline,	marginal	elevation.	1 mm.
Au:	2	»	»	»		
Cd:	3	»	»	»		
Hg:	4	»	»	»		
As:	7	»	diffuse	»		
Sb:	4	»	sharp	»	, marginal elevation	1 mm.
Bi:	1	»	»	»		
Te:	2	»	diffuse	»	with 7 mm. weak growth outside it,	
					followed by 2 mm. of increased growth. After 4 days	
					there are 4 mm. of brown colour in the colonies.	
Co:	2	»	sharp	borderline.	After 4 days 4 mm. red tint around	
					the metal, after 6 days 7 mm.	

XXIV. *Bacterium vulgare* X₁₉.

Cu:	2 mm,	sharp	borderline,	marginal	elevation 2 mm.	After 4 days	
					8 mm. of violet colour around the metal.		
Ag:	1	»	sharp	borderline.			
Au:	1	»	»	»			
Cd:	5	»	diffuse	»			
Hg:	3	»	sharp	»			
As:	10	»	diffuse	»			
Sb:	5	»	diffuse	»			
Bi:	1	»	sharp	»			
Te:	7	»	»	»	, marginal elevation 1 mm.	After 2	
					days 6 mm. of brown colour in the culture.		
Co:	3	»	sharp	borderline	marginal elevation 1 mm.	After 4	
					days 4 mm. red tint around the metal.		

XXV. *Bacillus mycoides*.

Cu:	2 mm,	sharp	borderline.				
Ag:	2	»	»	»	, marginal elevation 1 mm.		
Au:	1	»	»	»	»	»	0.5 mm.
Cd:	4	»	diffuse	»			
Hg:	8	»	»	»			
As:	15	»	»	»			
Sb:	10	»	sharp	»	, marginal elevation 2 mm.		
Bi:	2	»	»	»			
Te:	6	»	»	»	. After 4 days 6 mm. of brown colour		
					in the colonies.		

Co: 4 mm. sharp borderline. After 6 days 8 mm. red tint around the metal.

XXVI. Bacillus subtilis.

Cu: 2.5 mm, sharp borderline.

Ag: 4 » » » . After 4 days 4 mm. of brown colour around the metal, after 8 days 7 mm.

Au: 1 » sharp borderline.

Cd: 8 » » »

Hg: 9 » diffuse »

As: 6 » » »

Sb: 12 » » »

Bi: 2 » sharp »

Te: 9 » diffuse »

Co: 5 » sharp » . After 4 days a red tint of 4 mm. around the metal, after 8 days 9 mm.

XXVII. Bacillus vulgaris.

Cu: 2 mm, sharp borderline.

Ag: 2 » » »

Au: 1 » » »

Cd: 6 » » »

Hg: 3 » » »

As: 8 » diffuse »

Sb: 3 » sharp »

Bi: 2 » » »

Te: 4 » » » . After 6 days 9 mm. of brown colour in the colonies.

Co: 3 » sharp borderline, marginal elevation 1 mm. After 6 days 10 mm. of red colour around the metal.

XXVIII. Bacillus mesentericus.

Cu: 2 mm, sharp borderline.

Ag: 2 » » » , after 1 day marginal elevation 1 mm. After 4 days 4 mm. of brown colour around the metal, after 8 days 6 mm.

Au: 0.5 » sharp borderline.

Cd: 6 » » » , after 4 days, marginal elevation 2 mm.

Hg: 5 » » »

As: 12 mm, sharp borderline, after 4 days, marginal elevation 2 mm.
 Sb: 10 » » » » » » 2 »
 Bi: 0.5 » » »
 Te: 5 » » » , after 1 day, marginal elevation 1 mm.
 Co: 3 » » » . After 4 days, 7 mm. of red colour
 around the metal.

XXIX. *Bacillus teres*.

Cu: 2 mm, sharp borderline.
 Ag: 2 » » » , marginal elevation 1 mm. After
 4 days 3 mm. of brown colour around the metal, after
 8 days 5 mm.
 Au: 1.5 » . sharp borderline.
 Cd: 7 » diffuse »
 Hg: 12 » » »
 As: 10 » » »
 Sb: 8 » sharp » marginal elevation 2 mm.
 Bi: 1.5 » » »
 Te: 7 » diffuse » , the brown colour of the colonies is
 4 mm. deep, after 4 days 6 mm, after 6 days 9 mm.
 Co: 2 » sharp borderline. After 4 days 7 mm. red tint around
 the metal, after 6 days 10 mm.

XXX. *Bacillus asterosporus*.

Cu: 1 mm, sharp borderline.
 Ag: 4 » » »
 Au: 2 » » »
 Cd: 6 » » »
 Hg: 10 » » »
 As: 10 » diffuse »
 Sb: 14 » sharp »
 Bi: 3 » » » , 1 mm. marginal elevation, after 4 days
 it is 2 mm.
 Te: 8 » sharp borderline.
 Co: 4 » » » . After 4 days 6 mm. red tint around
 the metal, after 8 days 9 mm.

XXXI. *Vibrio cholerae*.

Cu: 4 mm, sharp borderline.
 Ag: 2 » » »

Au:	mm,	sharp	borderline,	marginal	elevation	1 mm.
Cd:	7	»	»	»		
Hg:	3	»	»	»	,	marginal elevation 1 mm.
As:	15	»	diffuse	»		
Sb:	10	»	sharp	»		
Bi:	1	»	»	»		
Te:	5	»	diffuse	»	,	outside it 5 mm. of weak growth, then comes a sharp borderline with increased growth.
Co:	3	»	sharp	borderline.		

XXXII. *Vibrio cholerae* (El Tor).

Cu:	4 mm,	sharp	borderline.	After 4 days	1 mm. light green rim around the metal and a violet circle 6 mm. wide.
Ag:	4	»	sharp	borderline,	after 3 days marginal elevation 1 mm.
Au:	1	»	»	»	
Cd:	6	»	»	»	, after 3 days marginal elevation 2 mm.
Hg:	4	»	»	»	
As:	11	»	diffuse	»	
Sb:	9	»	sharp	»	, after 3 days marginal elevation 1.5 mm.
Bi:	1	»	»	»	
Te:	4	»	»	»	, outside it 10 mm. of weak growth, followed by 2 mm. of increased growth. The colonies in the area of increased growth become brown in 4 days.
Co:	5	»	sharp	borderline,	marginal elevation 1 mm. After 4 days a red tint of 8 mm. around the metal.

XXXIII. *Vibrio proteus* (Finkler-Priori).

Cu:	2 mm,	sharp	borderline.	After 1 day	3 mm. of violet colour around the metal, after 4 days 6 mm, after 6 days 9 mm.
Ag:	3	»	sharp	borderline.	
Au:	3	»	»	»	
Cd:	4	»	»	»	, marginal elevation 0.5 mm, which grows in 4 days to 1 mm.
Hg:	3	»	sharp	borderline,	after 3 days, marginal elevation 1 mm, after 6 days 2 mm.
As:	10	»	diffuse	borderline.	
Sb:	8	»	sharp	»	, after 4 days marginal elevation 1 mm.
Bi:	2	»	»	»	

- Te: 5 mm, sharp borderline, followed by 9 mm. of weak growth, colonies brown-coloured to the extent of 10 mm.
 Co: 4 » sharp borderline, marginal elevation 2 mm. After 4 days 5 mm. red tint around the metal, after 6 days 7 mm.

XXXIV. *Vibrio albensis*.

- Cu: 3 mm, sharp borderline. After 3 days 1 mm. light green rim around the metal and 5 mm. of violet colour.
 Ag: 1 » sharp borderline.
 Au: 1 » » »
 Cd: 4 » » » , marginal elevation 0.5 mm.
 Hg: 3 » » »
 As: 8 » » »
 Sb: 5 » » »
 Bi: 1 » » »
 Te: 4 » » » , followed by 6 mm. of weak growth. After 3 days a marginal elevation of 0.5 mm. in the border line. After 6 days 9 mm. of brown colour in the colonies.
 Co: 5 » sharp borderline, after 2 days marginal elevation 2 mm. and 5 mm. red tint around the metal, after 6 days the latter is 8 mm.

XXXV. *Vibrio aquatilis* (Rudolfs-Hospital).

- Cu: 2 mm, sharp borderline.
 Ag: 2 » » » , marginal elevation 0.5 mm.
 Au: 1 » » » , after 2 days marginal elevation 0.5 m.
 Cd: 3 » » » » 3 » » » 2 »
 Hg: 3 » » » » 3 » » » 1 »
 As: 13 » diffuse »
 Sb: 11 » » »
 Bi: 1 » sharp »
 Te: 5 » diffuse » , then follows 6 mm. of weak growth with 2 mm. marginal elevation. After 6 days the colonies become brown from the marginal elevation outwards, and the intensity of the colour increases during the 3 following days.
 Co: 2 » sharp borderline, marginal elevation 1 mm. After 4 days 6 mm. red tint around the metal.

XXXVI. *Corynebacterium pseudodiphtheriticum*.

Cu:	1 mm,	sharp	borderline.	After 4 days 6 mm. of brown colour around the metal, after 8 days 10 mm.
Ag:	3	»	sharp	borderline, marginal elevation 1 mm.
Au:	0.5	»	»	»
Cd:	5	»	»	», marginal elevation 0.5 mm.
Hg:	2	»	»	»
As:	6	»	diffuse	»
Sb:	6	»	sharp	»
Bi:	1	»	»	»
Te:	4	»	»	»
Co:	4	»	»	», marginal elevation 2 mm. After 4 days 5 mm. red tint around the metal.

XXXVII. *Mycobacterium (phlei) flavum*, cultivated by Murto.

Cu:	4 mm,	sharp	borderline.	
Ag:	2.5	»	»	»
Au:	1.5	»	»	»
Cd:	20	»	diffuse	», 4 mm. of metallic lustre in the colonies
Hg:	37	»	»	»
As:	26	»	»	»
Sb:	24	»	»	»
Bi:	3	»	sharp	», marginal elevation 1 mm.
Te:	5	»	»	», 10 mm. of brown colour in the colonies; the intensity of the colour increases during the 3 following days.
Co:	3	»	sharp	borderline, marginal elevation 1 mm. After 4 days 4 mm. red tint around the metal, after 6 days 7 mm.

XXXVIII. *Mycobacterium (phlei) rubrum*; cultivated by Murto.

Cu:	6 mm,	sharp	borderline,	marginal elevation 1 mm.
Ag:	3	»	»	»
Au:	0.5	»	»	»
Cd:	20	»	diffuse	»
Hg:	36	»	»	»
As:	22	»	»	»
Sb:	32	»	»	»
Bi:	3	»	sharp	»

- Te: 4 mm, sharp borderline, After 4 days 5 mm. of brown colour in the colonies, after 8 days 12 mm.
 Co: 3 » sharp borderline. After 4 days 4 mm. red tint around the metal, after 6 days 7 mm.

XXXIX. *Actinomyces chromogenes*.

- Cu: 4 mm, diffuse borderline.
 Ag: 4 » sharp » , marginal elevation 1 mm.
 Au: 2 » » » » 0.5 mm.
 Cd: 3 » bacteria free zone, 11 mm. area with weak growth, sharp borderline and marginal elevation 2 mm. Around the element 9 mm. of metallic lustre. The metallic lustre and the marginal elevation increase during the two following days.
 Hg: 2 » sharp borderline, marginal elevation 2 mm. of a slightly brown colour; after 4 days all colonies are brown.
 As: 15 » diffuse borderline. After 3 days increased growth at the border of the culture.
 Sb: 15 » sharp borderline, marginal elevation 1 mm.
 Bi: 3 » » » , marginal elevation 1 mm.
 Te: 5 » » » , then follow 4 mm. of weak growth with brown colonies, encircled by 3 mm. dark brown marginal elevation with a slightly raised centre; in the brown coloured area and marginal elevation appears a metallic lustre, which increases after 1 day.
 Co: 4 » sharp borderline, followed by 2 mm. of weak growth, outside it 4 mm. light grey, slightly elevated border-wall. After 2 days a red tint appears round the metal; the borderwall is also coloured, after 4 days 6 mm. of red colour appears outside it, after 6 days 9 mm; measured from the metal the red tint extends 19 mm.

XL. *Leptothrix*. *Sp. i*.

- Cu: 1.5 mm, sharp borderline. 1 mm. light green rim around the metal, encircled by 4 mm. of violet colour; after 4 days the colour extends 8 mm, after 8 days 11 mm.
 Ag: 2 » sharp borderline, marginal elevation 1 mm.
 Au: 0.5 » » »
 Cd: 5 » » »

- Hg: 5 mm, sharp borderline.
- As: 10 » diffuse » , outside it 8 mm. of weak growth, with diffuse margin.
- Sb: 5 » diffuse borderline, followed by 6 mm. of weak growth, which joins the normal growth without any clearly visible border.
- Pi: —, the colonies extend to the metal.
- Te: 10 » sharp borderline, followed by 4 mm. of weak growth with 2 mm. brown marginal elevation. After 4 days the brown colour extends 6 mm.
- Co: 4 » sharp borderline. After 6 days 8 mm. red tint around the metal.

On closer investigation of the element's injurious effect on cultures the following is revealed. Cu caused a sharp limitation in the visible bacteria culture with all bacteria tested with the exception of No. XII which grew into the metal, and XIX and XXXIX which showed a diffuse border line in the culture. I, VII, XXIV and XXXVIII formed a marginal elevation. Considering Nos. I, III, VIII, XI, XII, XIII, XVI, XVII, XVIII, XIX, XXI, XXII, XXIII, XXIV, XXXII, XXXIII, XXXIV and XL a light-green border of varying width, a violet tint in the agar, or only the latter, was visible round the metal. Brown dye round the metal was observed with Nos. VII, XII and XXXVI.

With all bacteria under test there formed a sharp limitation of bacteria cultures round Ag, and a marginal elevation as well with Nos. I, III, X, XVII, XVIII, XIX, XXI, XXII, XXIII, XXV, XXVIII, XXIX, XXXII, XXXV, XXXVI, XXXVIII, XXXIX and XL. Brown dye round the metal was visible with Nos. VI, VII, XI, XII, XIII, XVII, XIX, XXI, XXII, XXVI, XXVIII and XXIX.

The colonies growing round Au had a sharp border line and a marginal elevation took place with I, III, VI, VII, IX, X, XIII, XV, XVI, XVII, XIX, XXV, XXXI, XXXV and XXXIX. Brown dye round the metal was observed with Nos. I and XIX.

Under the influence of Cd the colonies acquired a sharp outline with Nos. I, II, III, IV, V, VII, VIII, XI, XII, XIII, XVII, XVIII, XIX, XXI, XXIII, XXVI, XXVII, XXVIII, XXX, XXXI, XXXII, XXXIII, XXXIV, XXXV, XXXVI, XXXIX and XL. Nos. III, IV, V, VII, XXVIII, XXXII, XXXIII, XXIV, XXXV,

XXXVI, XXXIX formed a marginal elevation. With VII, XXXVII and XXXIX a metallic gleam was observed round the element.

Hg caused a sharp limitation of the cultures with Nos. III, V, VII VIII, IX, X, XI, XII, XIII, XIV, XV, XVI, XVII, XVIII, XIX, XX, XXI, XXII, XXIII, XXIV, XXVII, XXVIII, XXX, XXXI, XXXII, XXXIII, XXXIV, XXXV, XXXVI, XXXIX and XL. In addition to this a marginal elevation took place with Nos. III, VIII, IX, X, XII, XVI, XVII, XVIII, XXI, XXXI, XXXIII, XXXV and XXXIX. The rest of the micro-organisms under test showed but a diffuse borderline between the culture and the bacteria-free zone.

With the exception of XVII, XVIII, XXI, XXVIII and XXXIV the borderline round As was diffuse. A marginal elevation formed with XVII, XVIII and XXVIII.

Sb produced a sharp outline in the colonies with I, III, VI, VII, VIII, IX, XIII, XVII, XX, XXI, XXIII, XXV, XXVII, XXVIII, XXIX, XXX, XXXI, XXXII, XXXIII, XXXIV, XXXVI and XXXIX. A marginal elevation was formed with Nos. VI, VII, XVII, XX, XXIII, XXV, XXVIII, XXIX, XXXII, XXXIII and XXXIX. The remaining micro-organisms showed but a diffuse outline of cultures.

Bi gave a sharp outline to all bacteria cultures save two and a marginal elevation with I, XIX, XXI, XXX, XXXVII and XXXIX. Nos. XII and XL grew into the metal.

A sharp outline was observed in the cultures round Te when the following bacteria were used: II, III, IV, V, VI, VII, VIII, IX, X, XII, XIII, XIV, XV, XVI, XVII, XVIII, XIX, XX, XXII, XXIV, XXV, XXVII, XXVIII, XXX, XXXII, XXXIII, XXXIV, XXXVI XXXVII, XXXVIII, XXXIX and XL. A marginal elevation was formed with IV, VII, VIII, X, XII, XIII, XVI, XVII, XVIII XXIV, XXVIII, XXXIV, XXXV, XXXIX and XL. It often happens that a brown dye is formed round this metal in the cultures. The majority of bacteria which were tested showed this brown dye and only the colonies of Nos. IV, XIV, XV, XVI, XIX, XX, XXVI, XXVIII, XXX, XXXI and XXXVI failed to produce it.

The influence of Co produced a sharp outline of bacteria cultures; No. V alone had a diffuse borderline. Cultures of Nos. I, II, III, IV, VII, VIII, IX, XI, XII, XVII, XVIII, XIX, XXI, XXIV, XXVII, XXXII, XXXIII, XXXIV, XXXV, XXXVI, XXXVII and XXXIX formed a marginal elevation. A red dye was observed

to have spread round the metal except in the case of bacteria IV, V, XVII, XIX and XXXI.

In general the elements of group I of the periodic system, Cu, Ag and Au, created but small bacteria free regions. With 17 bacteria Ag had a larger bacteria-free region round it than Cu, which again had a larger zone free from bacteria with but 12 bacteria. With the others the bacteria free zone was of the same size. The bacteria free zone round Au proved to be very small. In this group no certain relation could be found between the atomic weight and growth impeding effect.

In group II, with 21 micro-organisms, Cd created a larger bacteria free zone, Hg with 15 micro-organisms and with 4 such the bacteria free regions were equally large. In this group the element with the lower atomic weight had the stronger harmful effect.

The elements of group V produced the largest bacteria free zone with the bacteria used for these tests. Mycobacteria constituted an exception in that Hg produced with them a larger bacteria zone than As and Sb. Bi gave but a very limited bacteria free zone. With 30 micro-organisms As produced a larger bacteria free region than Sb, which latter had a larger free zone with 7 bacteria and an equally large zone with three. The growth impeding effect in this group decreased in accordance with the rising atomic weights of elements. Te usually caused the formation of a large region devoid of bacteria. The effect of Co was somewhat more pronounced than that of elements in group I. The foregoing remarks concern the growth resisting effect of elements.

In the tables appearing below mention is made concerning the span of life evidenced by various micro-organisms under the influence of different elements; with other words: the tables show the germicidal property of elements in agar cultures.

I. Sarcina flava.

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	+++	+++	+++	++	++	—	—	—	—	—	—	—	—	—
Ag	+++	+++	+++	+++	++	++	++	++	—	—	—	—	—	—
Au	+++	+++	+++	+++	+++	+++	++	++	++	++	++	++	—	—
Cd	+++	+++	+++	+++	+++	++	++	++	++	++	—	—	—	—
Hg	+++	+++	+++	+++	+++	++	++	++	+	—	—	—	—	—
As	+++	+++	+++	+	—	—	—	—	—	—	—	—	—	—
Sb	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	—
Bi	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++
Te	+++	+++	+++	+++	+++	+++	++	++	++	++	++	—	—	—
Co	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++

II. Micrococcus candicans.

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	—	—
Ag	+++	+++	+++	+++	+++	+++	++	++	++	—	—	—	—	—
Au	+++	+++	+++	+++	+++	+++	++	++	++	++	++	++	—	—
Cd	+++	+++	+++	+++	+++	+++	++	++	+	+	—	—	—	—
Hg	+++	+++	++	++	—	—	—	—	—	—	—	—	—	—
As	+++	+++	+	—	—	—	—	—	—	—	—	—	—	—
Sb	+++	+++	+++	+++	++	+	—	—	—	—	—	—	—	—
Bi	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	+	+	—
Te	+++	+++	+++	+++	++	++	+	—	—	—	—	—	—	—
Co	+++	+++	+++	+++	+++	+++	++	+	+	+	—	—	—	—

III. Micrococcus (pyogenes) aureus.

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	+++	+++	+++	+++	+++	++	++	—	—	—	—	—	—	—
Ag	+++	+++	+++	+++	+++	—	—	—	—	—	—	—	—	—
Au	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	—	—
Cd	+++	+++	++	++	++	++	++	++	++	+	—	—	—	—
Hg	+++	+++	+++	+++	+++	++	++	—	—	—	—	—	—	—
As	+++	+++	++	++	+	—	—	—	—	—	—	—	—	—
Sb	+++	+++	++	++	+++	++	++	++	—	—	—	—	—	—
Bi	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+	—	—	—
Te	+++	+++	+++	+++	+++	+++	+++	++	++	—	—	—	—	—
Co	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	—	—

VII. *Bacillus abortus* (typ. human. Bang)

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	+++	+++	+++	+++	+++	++	-							
Ag	+++	+++	+++	+++	+++	+++	++	++	++	-	-	-	-	-
Au	+++	+++	+++	+++	+++	+++	+++	+	+	+	+	+	+	+
Cd	+++	+++	+++	+++	+++	+++	+++	+	+	+	+	+	+	+
Hg	+++	+++	++	++	++	-	-	-	-	-	-	-	-	-
As	+++	++	++	-	-	-	-	-	-	-	-	-	-	-
Sb	+++	+++	+++	+++	+++	+++	++	+	+	+	+	+	+	+
Bi	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+	-	-
Te	+++	+++	+++	+++	++	++	++	-	-	-	-	-	-	-
Co	+++	+++	+++	+++	+++	+++	+++	++	++	+	-	-	-	-

VIII. *Bacillus abortus* (typ. bovin).

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	+++	++++	++++	+++	++	++	++	-	-	-	-	-	-	-
Ag	+++	++++	++++	++++	++++	++++	++	++	--	-	-	-	-	-
Au	+++	++++	++++	++++	++++	++++	++++	++++	++	+	+	+	+	-
Cd	+++	++++	++++	+++	+++	+++	+++	++	++	-	-	-	-	-
Hg	+++	++++	++++	+++	+++	++	++	-	-	-	-	-	-	-
As	+++	++++	++++	++++	++	-	-	-	-	-	-	-	-	-
Sb	+++	++++	++++	++++	+++	++	++	-	-	-	-	-	-	-
Bi	+++	++++	++++	+++	+++	+++	+++	+++	+	+	+	+	-	-
Te	+++	++++	++++	++++	+++	++	++	+	-	-	-	-	-	-
Co	+++	++++	++++	+++	+++	+++	+++	+++	++	++	++	++	+	-

IX. *Bacterium (lactis) viscosum*.[illegible]

XIII. *Bacterium paratyphi B.*

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ag	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Au	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cd	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hg	+	+	+	+	+	+	+	+	+	+	+	+	+	+
As	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sb	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bi	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Te	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Co	+	+	+	+	+	+	+	+	+	+	+	+	+	+

XIV. *Bacterium (typhi) murium.*

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ag	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Au	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cd	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hg	+	+	+	+	+	+	+	+	+	+	+	+	+	+
As	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sb	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bi	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Te	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Co	+	+	+	+	+	+	+	+	+	+	+	+	+	+

XV. *Bacterium ratti.*

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ag	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Au	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cd	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hg	+	+	+	+	+	+	+	+	+	+	+	+	+	+
As	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sb	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bi	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Te	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Co	+	+	+	+	+	+	+	+	+	+	+	+	+	+

XIX. *Bacterium prodigiosum*.

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	++	+	+++	++	-	-	-	-	-	-	-	-	-	-
Ag	++++	+	+++	+++	++	++	++	-	-	-	-	-	-	-
Au	++++	+	++	++	++	++	++	++	++	++	++	++	++	++
Cd	++++	+	+++	+++	++	++	++	++	++	++	++	++	++	++
Hg	++++	+	++	++	-	-	-	-	-	-	-	-	-	-
As	++	+	++	++	++	-	-	-	-	-	-	-	-	-
Sb	+++	++	++	++	++	++	++	++	++	++	++	++	++	++
Bi	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	++
Te	+++	+++	+++	+++	+++	+++	+++	+++	++	++	-	-	-	-
Co	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++

XX. *Pseudomonas caerulea*.

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	++	+	-	-	-	++	++	-	-	-	-	-	-	-
Ag	-	+	+	-	-	++	-	-	-	-	-	-	-	-
Au	+	+	+	+	-	+++	+++	+++	+++	+++	++	++	+	+
Cd	+	+	+	-	-	++	++	++	-	-	-	-	-	-
Hg	++	+	+	+	+	++	++	-	-	-	-	-	-	-
As	+	+	-	+	-	-	-	-	-	-	-	-	-	-
Sb	++	-	+	+	+	+++	+++	+++	++	++	++	++	-	-
Bi	++	+	+	+	+	+++	+++	+++	+++	+++	+++	+++	++	++
Te	++	+	+	+	+	+++	+++	+++	++	++	++	-	-	-
Co	+++	+	+	+	+	+++	+++	+++	+++	+++	++	++	++	++

XXI. *Pseudomonas pyocyanea*.

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	+++	+++	++	++	—	—	—	—	—	—	—	—	—	—
Ag	+++	+++	+++	++	++	—	—	—	—	—	—	—	—	—
Au	+++	+++	+++	+++	+++	++	++	++	++	++	++	++	++	++
Cd	+++	+++	+++	+++	+++	++	++	++	++	++	++	++	++	++
Hg	+++	+++	++	++	++	++	++	—	—	—	—	—	—	—
As	+++	+++	++	++	++	—	—	—	—	—	—	—	—	—
Sb	+++	+++	++	++	++	++	++	++	++	++	++	++	++	++
Bi	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Te	+++	+++	+++	+++	+++	+++	++	++	++	—	—	—	—	—
Co	+++	+++	+++	+++	+++	+++	+++	++	++	+	+	—	—	—

XXVIII. *Bacillus mesentericus*.

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	+++	+++	+++	+++	+++	+++	++	—	—	—	+	—	—	—
Ag	+++	+++	+++	+++	+++	++	++	++	++	—	—	—	—	—
Au	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++
Cd	+++	+++	+++	+++	+++	++	++	++	++	++	—	—	—	—
Hg	+++	+++	+++	+	+	—	—	—	—	—	—	—	—	—
As	+++	+++	+	—	—	—	—	—	—	—	—	—	—	—
Sb	+++	+++	+++	+++	+++	—	—	—	—	—	—	—	—	—
Bi	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++
Te	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++
Co	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++

XXIX. *Bacillus teres*.

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	+++	+++	+++	+++	+++	+	+	—	—	—	—	—	—	—
Ag	+++	+++	+++	++	++	—	—	—	—	—	—	—	—	—
Au	+++	+++	+++	+++	+++	++	++	+	+	+	+	+	—	—
Cd	+++	+++	+++	+++	+++	—	—	—	—	—	—	—	—	—
Hg	+++	+++	—	—	—	—	—	—	—	—	—	—	—	—
As	+++	—	—	—	—	—	—	—	—	—	—	—	—	—
Sb	+++	+++	+++	++	++	—	—	—	—	—	—	—	—	—
Bi	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	—
Te	+++	+++	+++	+++	+++	+++	++	++	—	—	—	—	—	—
Co	+++	+++	+++	+++	+++	+++	+++	++	++	++	+	+	—	—

XXX. *Bacillus asterosporus*.

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	+++	+++	+++	+++	+	+	—	—	—	—	—	—	—	—
Ag	+++	+++	+++	+++	++	++	++	—	—	—	—	—	—	—
Au	+++	+++	+++	+++	+++	+++	+++	++	++	+	+	+	—	—
Cd	+++	+++	+++	+++	+++	+	—	—	—	—	—	—	—	—
Hg	+++	+++	+	—	—	—	—	—	—	—	—	—	—	—
As	+++	+++	+++	+++	+++	—	—	—	—	—	—	—	—	—
Sb	+++	+++	+++	+++	+	+	—	—	—	—	—	—	—	—
Bi	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	+	+
Te	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	+	+
Co	+++	+++	+++	+++	+++	+++	+++	+	+	+	—	—	—	—

XXXI. *Vibrio cholerae*.

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	++	++	—	—	—	—	—	—	—	—	—	—	—	—
Ag	+++	++	++	++	—	—	—	—	—	—	—	—	—	—
Au	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+	—
Cd	++	++	++	—	—	—	—	—	—	—	—	—	—	—
Hg	+++	+++	++	++	++	+	+	—	—	—	—	—	—	—
As	+++	+++	++	+	—	—	—	—	—	—	—	—	—	—
Sb	+++	+++	+	+	+	+	+	—	—	—	—	—	—	—
Bi	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Te	+++	+++	+++	+++	+++	++	—	—	—	—	—	—	—	—
Co	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	—	—	—	—

XXXI. *Vibrio cholerae* (El Tor).

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	+++	+++	+	+	—	—	—	—	—	—	—	—	—	—
Ag	+++	+++	++	—	—	—	—	—	—	—	—	—	—	—
Au	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	—	—
Cd	+++	+++	++	++	—	—	—	—	—	—	—	—	—	—
Hg	+++	+++	+++	+++	+++	+++	+	—	—	—	—	—	—	—
As	+++	+++	+++	—	—	—	—	—	—	—	—	—	—	—
Sb	+++	+++	++	++	++	—	—	—	—	—	—	—	—	—
Bi	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Te	+++	+++	+++	+++	+++	++	+	+	—	—	—	—	—	—
Co	+++	+++	+++	+++	+++	+++	+++	+	+	+	—	—	—	—

XXXIII. *Vibrio proteus* (Finkler-Priori).

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	+++	+++	++	++	++	—	—	—	—	—	—	—	—	—
Ag	+++	+++	+++	+++	++	++	—	—	—	—	—	—	—	—
Au	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+	—	—
Cd	+++	+++	+++	+++	+++	++	++	++	—	—	—	—	—	—
Hg	+++	+++	+++	+++	++	++	++	—	—	—	—	—	—	—
As	+++	++	—	—	—	—	—	—	—	—	—	—	—	—
Sb	+++	+++	++	++	—	—	—	—	—	—	—	—	—	—
Bi	+++	+++	+++	+++	+++	++	++	++	+	+	+	+	+	+
Te	+++	+++	+++	++	++	++	++	+	+	—	—	—	—	—
Co	+++	+++	+++	+++	+++	++	++	++	—	—	—	—	—	—

XXXVII. *Mycobacterium (phlei) flavum* (cultivated by Murto).

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	+++	+++	++	++	—	—	—	—	—	—	—	—	—	—
Ag	+++	+++	+++	+++	++	—	—	—	—	—	—	—	—	—
Au	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	—	—	—
Cd	+++	+++	+++	—	—	—	—	—	—	—	—	—	—	—
Hg	++	++	++	—	—	—	—	—	—	—	—	—	—	—
As	++	++	++	—	—	—	—	—	—	—	—	—	—	—
Sb	++	++	++	++	—	—	—	—	—	—	—	—	—	—
Bi	+++	+++	+++	+++	+++	++	++	++	++	++	—	—	—	—
Te	+++	+++	+++	+++	+++	+++	+++	++	++	++	—	—	—	—
Co	+++	+++	+++	+++	+++	+++	+++	++	++	++	—	—	—	—

XXXVIII. *Mycobacterium (phlei) rubrum* (Cultivated by Murto).

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	+++	+++	++	++	++	++	—	—	—	—	—	—	—	—
Ag	+++	+++	+++	++	—	—	—	—	—	—	—	—	—	—
Au	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	—
Cd	+++	++	++	—	—	—	—	—	—	—	—	—	—	—
Hg	++	++	—	—	—	—	—	—	—	—	—	—	—	—
As	+++	++	—	—	—	—	—	—	—	—	—	—	—	—
Sb	+++	+++	++	++	—	—	—	—	—	—	—	—	—	—
Bi	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	—	—
Te	+++	+++	+++	+++	+++	+++	+++	++	++	—	—	—	—	—
Co	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	—	—	—

XXXIX. *Actinomyces chromogenes*.

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	++	++	++	++	++	++	—	—	—	—	—	—	—	—
Ag	+++	+++	+++	++	++	+	+	+	+	—	—	—	—	—
Au	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++
Cd	+++	+++	+++	+++	++	—	—	—	—	—	—	—	—	—
Hg	+++	+++	++	++	++	++	++	—	—	—	—	—	—	—
As	+++	+++	++	++	—	—	—	—	—	—	—	—	—	—
Sb	+++	+++	++	++	+	+	+	+	—	—	—	—	—	—
Bi	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++
Te	+++	+++	++	++	++	++	++	—	—	—	—	—	—	—
Co	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+	—	—

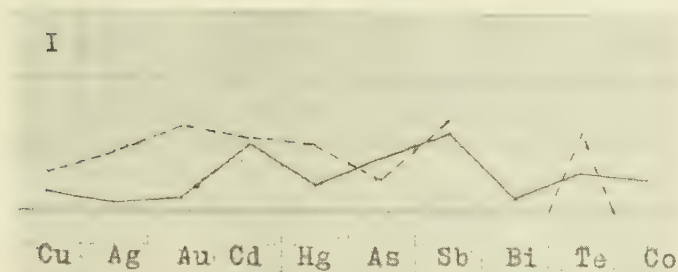
XL. *Leptothrix. Sp.i.*

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cu	+++	+++	+++	+++	+++	+++	+	+	—	—		—	—	—
Ag	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+	—	—	—
Au	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cd	+++	+++	+++	+++	+++	+++	+++	+++	+	+	—	—	—	—
Hg	+++	+++	+++	++	++	++	+	+	—	—	—	—	—	—
As	+++	++	++	+	+	+	—	—	—	—	—	—	—	—
Sb	+++	++	++	++	++	++	++	—	—	—	—	—	—	—
Bi	+	+	+	+	+	+	+	+	+	+	+	++	++	+
Te	+++	+++	+++	+++	+	+	+	—	—	—	—	—	—	—
Co	+++	+++	+++	+++	+++	++	++	++	++	++	++	++	—	—

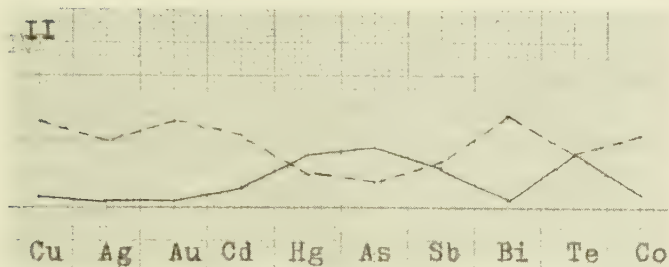
The above tables show that Hg and As had the strongest germicidal effect. Cu, Ag, Cd and Sb exercised a much weaker effect. Sb, although in most cases able to produce a considerable bacteria free zone, proved comparatively weak as a germicidal agent. Te also proved but mildly germicidal in a number of cases. Au and Bi had the weakest germicidal effect; it happened quite often that during the whole testing time they failed in their bactericidal effect on a number of bacteria. Under the continuous influence of elements the new colonies grew often smaller than the standard cultures. This shows that the micro-organisms were weakened by the elements. In the majority of cases bacteria showed weakened growth before finally succumbing; in some cases, however, they died quicker, especially when under the influence of Hg and As.

In the tables below is found a graphical illustration of the relation between the size of the bacteria free zone observed round the various elements, i.e. the injurious effect of the elements, and the time taken to destroy bacteria, viz. the germicidal effect of the elements. Where elements are concerned the size of the bacteria free zone is given in mm. in the vertical lines the different points have been connected to form a curve. The time of life with the different elements is illustrated in a similar manner, whereby the vertical lines show a day to the millimetre; the different points are connected by a broken line. In the absence of a germicidal effect during the period of the tests the line appears cut and the effect of a neighbouring element is shown by a broken line inside its column.

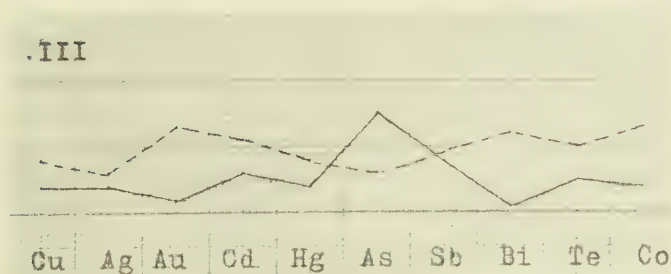
I. *Sarcina flava*.



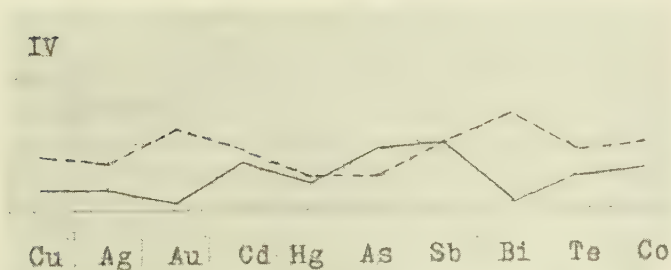
II. *Micrococcus candicans*.

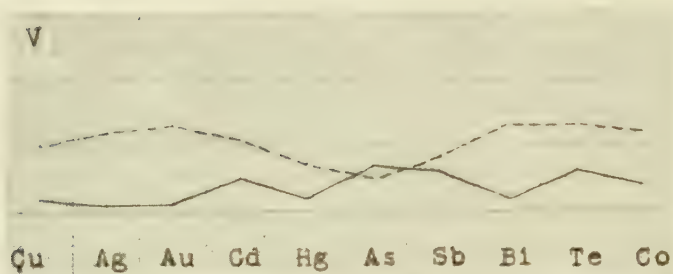
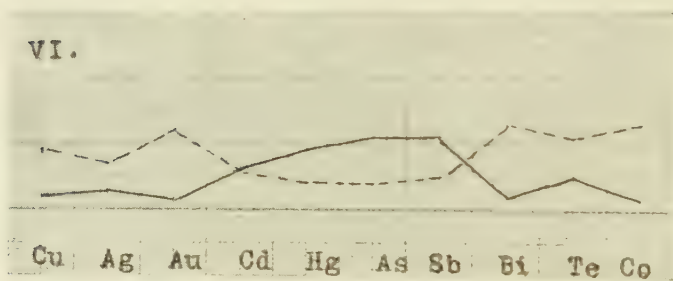
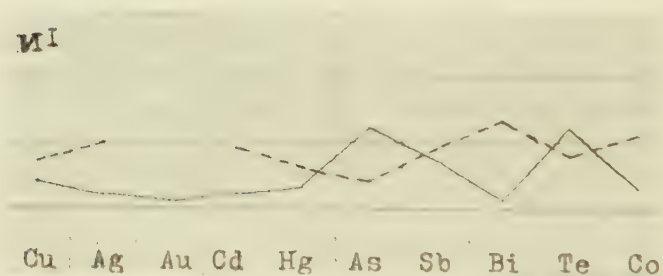
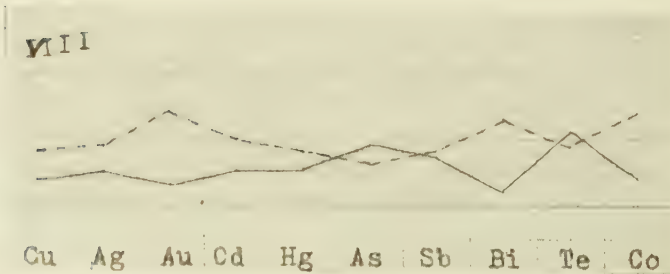


III. *Micrococcus (pyogenes) aureus*.

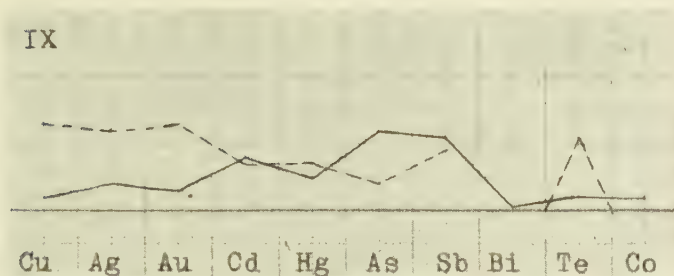


IV. *Micrococcus (pyogenes) citreus*.

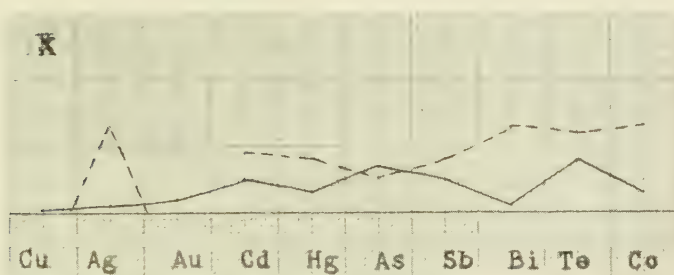


V. *Micrococcus (pyogenes) albus*.VI. *Micrococcus Freudenreichii*.VII. *Bacillus abortus* (typ. human, Bang).VIII. *Bacillus abortus* (typ. bovin).

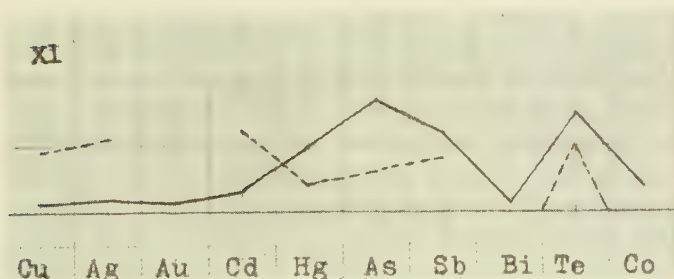
IX. *Bacterium lactis viscosum*.



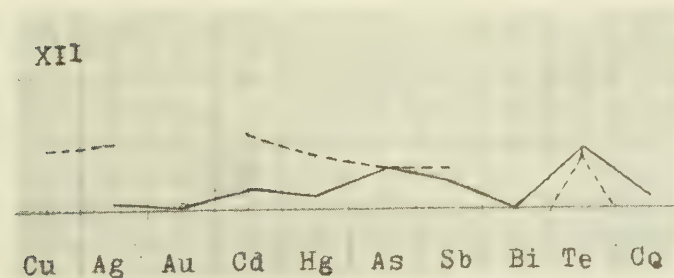
X. *Bacterium lactis amarum*.

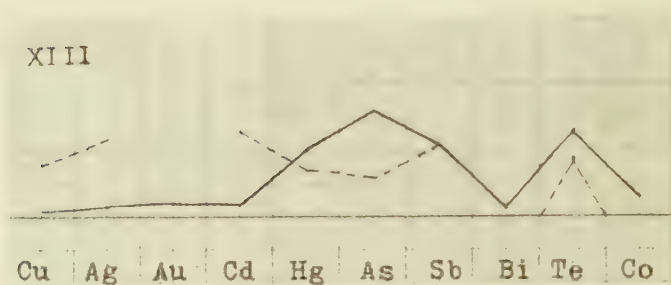
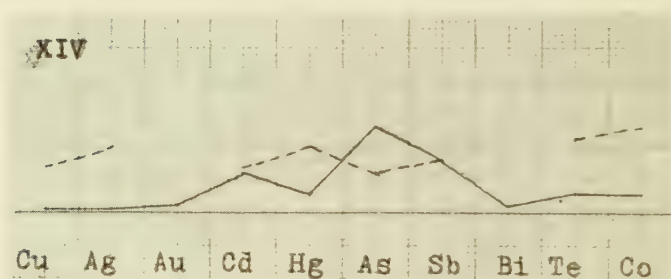
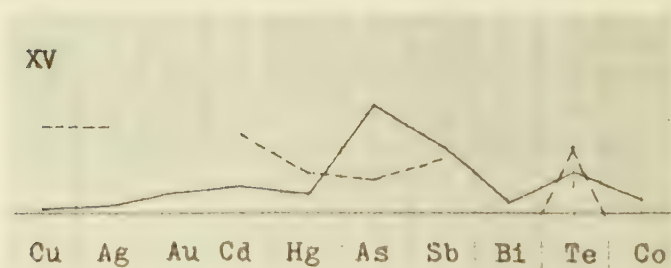
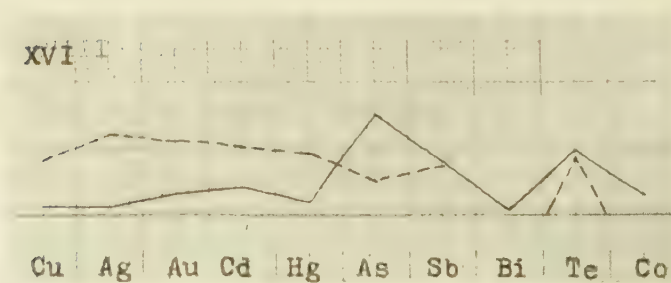


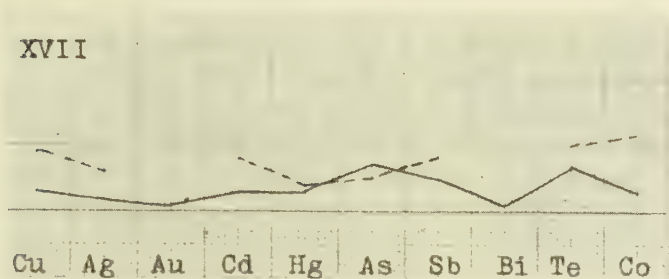
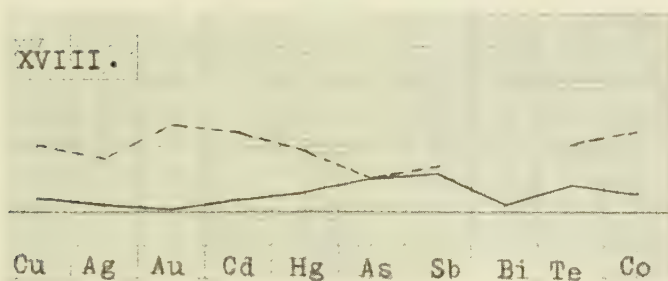
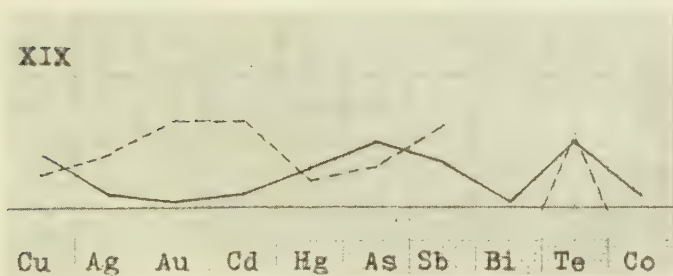
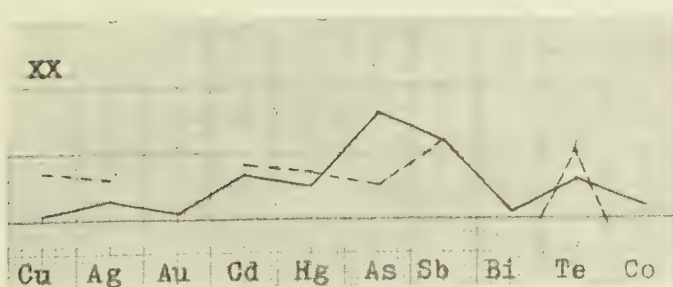
XI. *Bacterium typhi*.

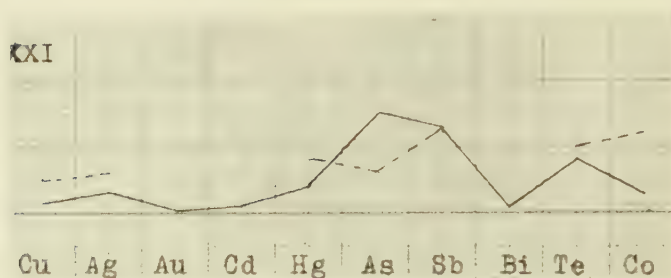
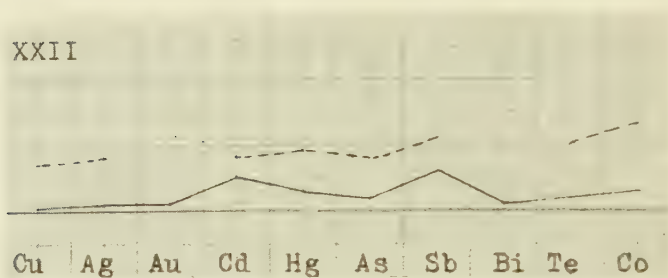
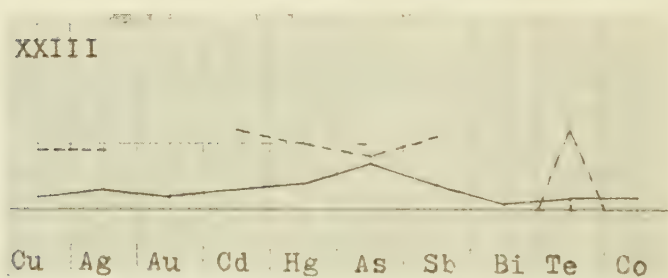
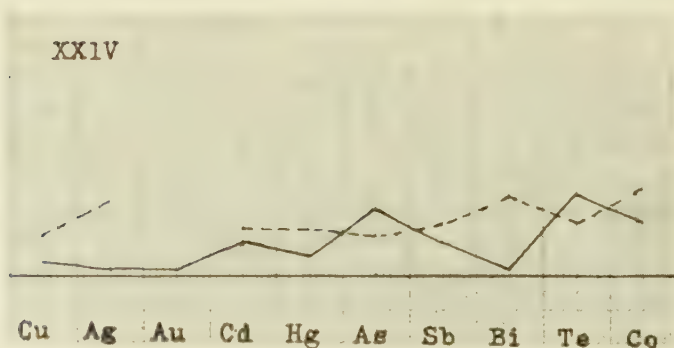


XII. *Bacterium (faecalis) alcaligenes*.

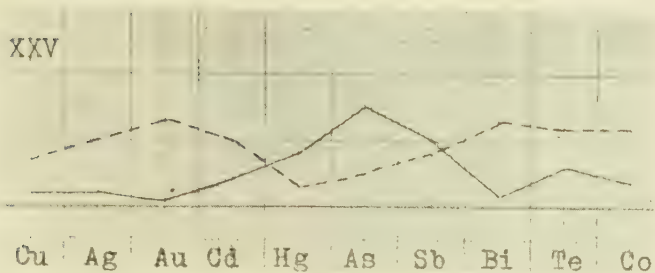


XIII. *Bacterium paratyphi B.*XIV. *Bacterium (typhi) murium.*XV. *Bacterium ratti.*XVI. *Bacterium (septicaemiae) murium.*

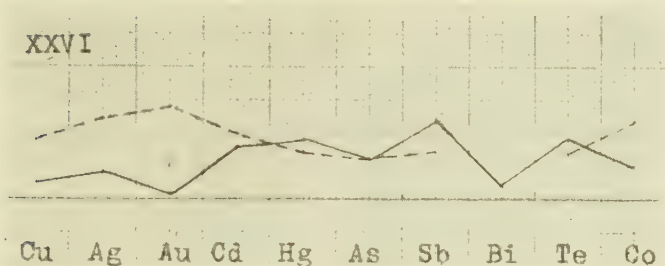
XVII. *Bacterium coli*.XVIII. *Bacterium coli*, home cultivated strain.XIX. *Bacterium prodigiosum*.XX. *Pseudomonae caerulea*.

XXI. *Pseudomonas pyocyanea*.XXII. *Pseudomonas putida*.XXIII. *Bacterium vulgare*. (*Proteus vulgaris*.)XXIV. *Bacterium vulgare* X₁₀.

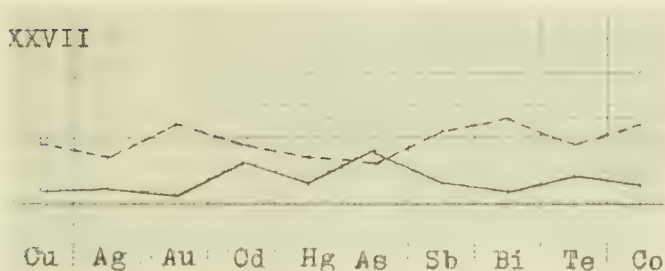
XXV. *Bacillus mycoides*.



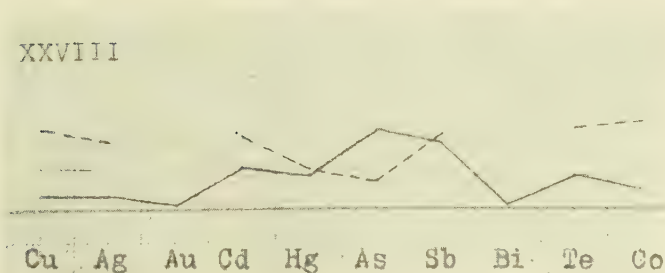
XXVI. *Bacillus subtilis*.



XXVII. *Bacillus vulgatus*.

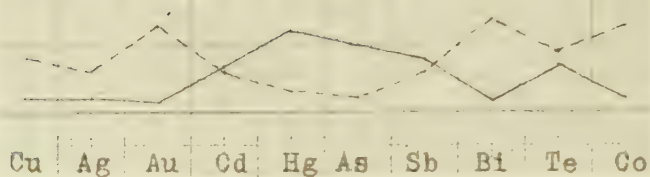


XXVIII. *Bacillus mesentericus*.

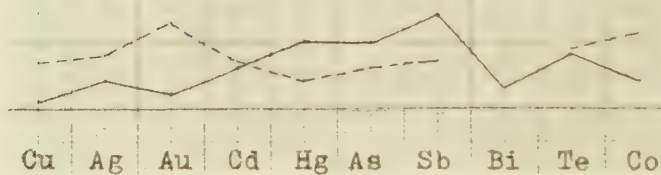


XXIX. *Bacillus teres*.

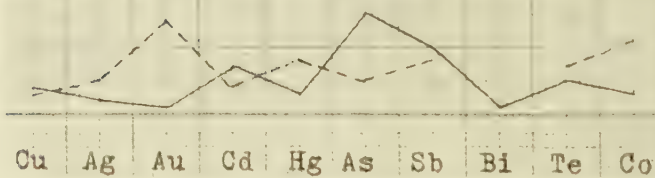
XXIX

XXX. *Bacillus asterosporus*.

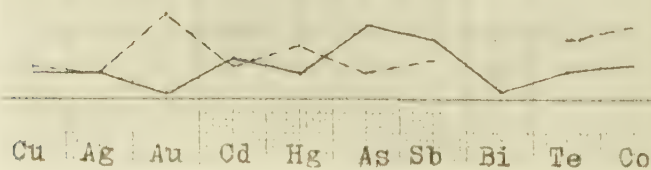
XXX

XXXI. *Vibrio cholerae*.

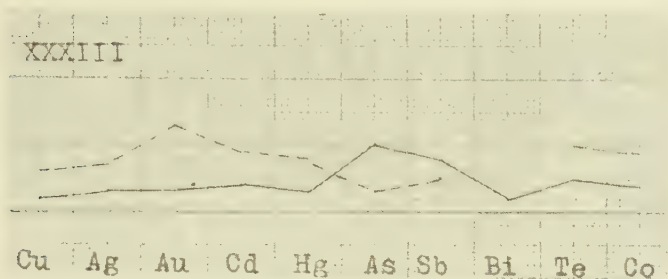
XXXI

XXXII. *Vibrio cholerae* (El Tor).

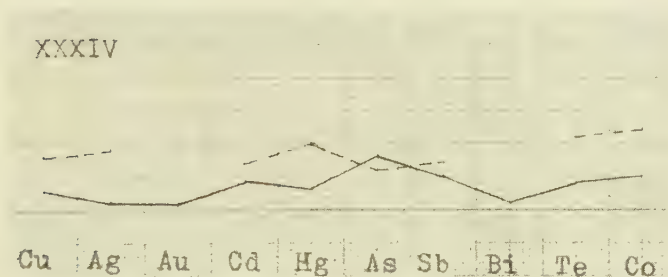
XXXII



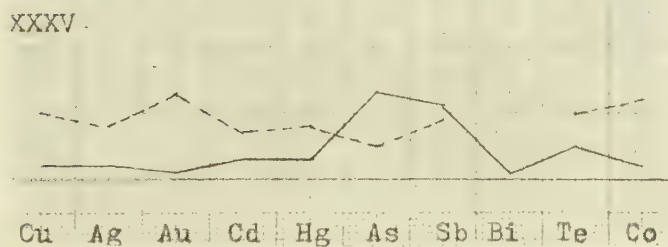
XXXIII. *Vibrio proteus* (Finkler-Priori).



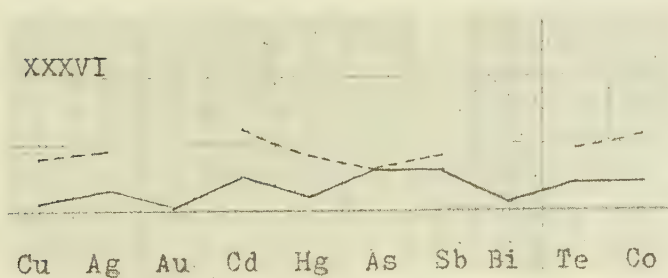
XXXIV *Vibrio albensis*.



XXXV. *Vibrio anguicilis* (Rudolfs Hospital).

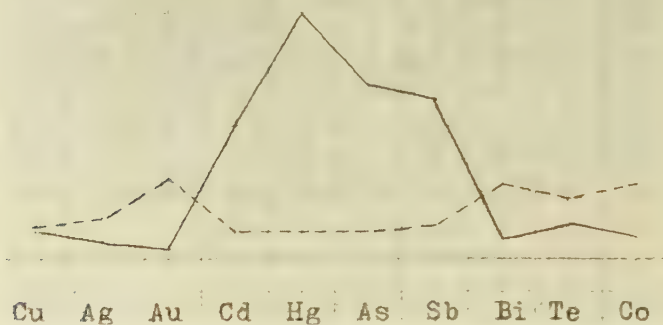


XXXVI. *Corynebacterium pseudodiphtheriticum*.



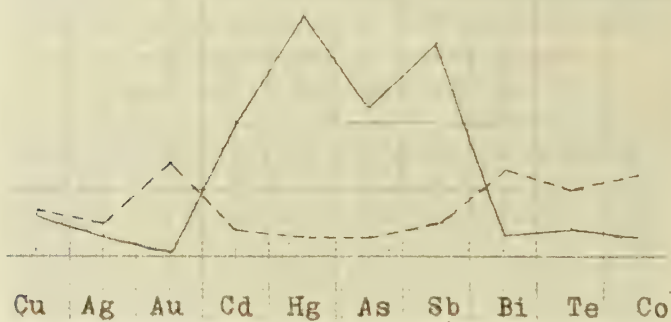
XXXVII. *Mycobacterium (phlei) flavum* (cultivated by Murto).

XXXVII



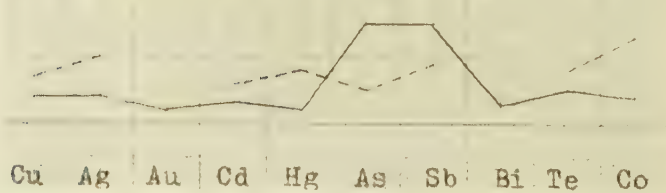
XXXVIII. *Mycobacterium (phlei) rubrum* (cultivated by Murto).

XXXVIII



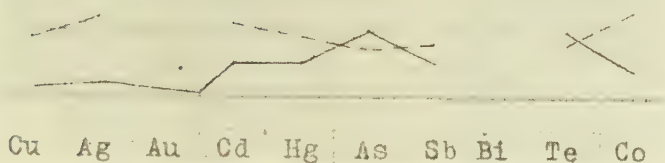
XXXIX. *Actinomyces chromogenes*.

XXXIX



XL. *Leptothrix. Sp. i.*

XL



The foregoing tables show that the greatest bactericidal effect is produced by elements with the largest bacteria free zone and vice versa. The former circumstance is apparent especially with Hg, As and even Sb, whilst Au and Bi, producing but a small bacteria free zone, have a considerably weaker bactericidal action. The curves denoted by a drawn out line rise in general from Cu, Ag and Au towards Cd, Hg and As; at the point of Sb there is usually a small depression which becomes still more pronounced at the point of Bi; the curve resumes its upward course at the point of Te. There is usually also another depression where Co stands. The two *Mycobacteria* constituted an exception in so far that Hg was the element to produce the largest bacteria free zone with them. The broken line, showing the curve, generally runs contrary to the other curve. Mildly bactericidal elements are usually those which produce but a small bacteria free zone, and those which produce a large one need a shorter time to destroy bacteria. The latter curve often breaks at Au and Bi which elements failed to destroy the corresponding micro-organism within the testing time.

The effect of the elements becomes distinct only when they are allowed to come into direct contact with the culture media; this effect remains unimpeded even if other obstacles are in the way. Such a case is found in figure 1. A culture of *Pseudomonas pyocyanea* is allowed to come under the influence of As and Sb which, according to what has been stated above, produce a large bacteria free zone. As 1 denotes the place where the As-piece has been placed on a thin bit of celluloid of the same size as the bottom surface of the element. The bacteria grows to the element. Another As-piece, a smaller one, lies on the agar itself and a bacteria free zone is clearly visible round the element. Sb 1 denotes the place where Sb lies on the agar in direct touch with it and covered up by a small glass

dome. At Sb 2 the element is also in direct contact with the culture media, but uncovered. In both cases the bacteria free regions are almost of the same size. Figure II shows the effect of Sb and Te on *B. paratyphi* B. At Sb 1 the metal is in direct touch with the agar and produces a distinct bacteria free zone; at Sb 2 the metal is insulated from the agar by a celluloid sheet with the result that the growth of bacteria is unimpeded. Lower down the two Te-pieces may be seen, they rest on celluloid and do not hamper the growth of bacteria. Figure III shows a culture of *Actinomyces chromogenes*. The effect of Sb is very strongly preventive; the marginal elevation round Co is exceedingly well defined. In figure IV a light green borderline is shown round Cu and outside it the agar is dyed dark violet. The same is the case in figure V where the metal is also in direct contact with the culture media. Figure VI shows no changes in the agar round Cu which rests on celluloid. Figure VII illustrates the effect of Te-powder on *Pseudomonas pyocyanea*. The bacteria free zone and the darkening of the colonies are clearly perceivable. In figure VIII the bacteria grows close up to the celluloid sheet supporting the Te-powder; no changes of colour are visible in the culture. With Te there is often a distinct brown dye and gleam of metal at the border of the culture. This may be seen in figure IX where the metal is acting on *Actinomyces chromogenes*. The gleam of metal is very clearly noticeable in figure X. Cd too often produces a metallic glitter, as a glance at figure XI will prove; in this case the metal is allowed to act on *Actinomyces chromogenes*.

In the aforementioned experiments all metals used are characterized by very definite injurious action. The elements Zn, Mo



Figure I.

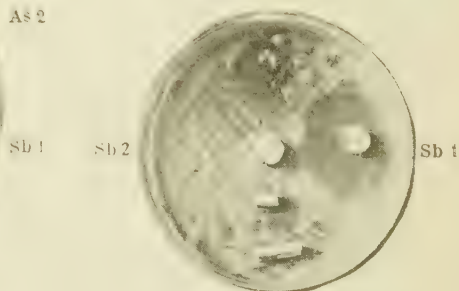


Figure II.



Figure III.

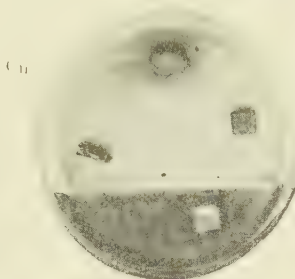


Figure IV.

and Tl have been used only to demonstrate their spreading in the culture media, when in direct touch with it. In figure XII the spread of blue dye around Mo may be seen quite distinctly. Figure XIII shows coagulation of the agar round Zn 1, this is not perceivable if Zn is insulated from the agar by a thin slip of celluloid, see Zn 2. Figure XIV shows white dots in the culture media round Tl; on investigation these dots showed contents of Tl which are unmistakable owing to the characteristic colour they give to a gas flame. Tl, when resting on a slip of celluloid, caused no changes in the agar, although the element became sheathed in grey.

These few examples prove beyond doubt that direct contact between the element and the culture media is necessary to produce a definite injurious effect. This lead to investigations in search of the possible existence of elements in the culture media itself.

The experiments were performed in the following way. Elements were allowed a fortnight in which to affect bacteria cultures as described above, *Pseudomonas pyocyanea* was usually chosen for this purpose. Usual chemical reactions could show the presence of only As in this way and it therefore became necessary to increase the number of elements in the different dishes up to 8—10 pieces each, all more or less of the same size. Of Hg were used three drops with a diameter of 7—10 mm. Cu and Co were not analyzed in this way. A fortnight later the elements were withdrawn, the agar vaporized on a water bath, the remainder was burned to ash and dissolved in either HCl or HNO₃, and an attempt was then made to show the presence of the elements by some kind of chemical reaction. Hg was analyzed in another

way. The elements, for the purpose of checking, were kept an equally long time in the dishes, but were placed on either glass or celluloid. In these cases no visible reaction took place.

Ag. The ash was dissolved in HNO_3 . With NaCl a weak opalescence of AgCl was obtained.

Au. The ash was dissolved in aqua regia which then was diluted with distilled water, the element could not be traced. According to Wo. OSTWALD minute quantities of Au may be detected in colloidal dispersion in a borax grain. The result was negative. The above mentioned scientist is of the opinion that this method is more sensitive than the spectroscope.

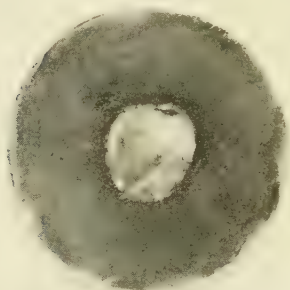


Figure V.

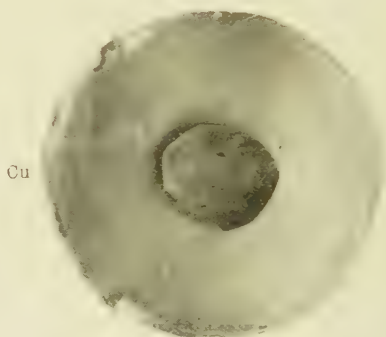


Figure VI.

Cd. The ash was dissolved in HCl and the solution was somewhat diluted with distilled water, H_2S caused a weak precipitate of cadmium sulphide.

Hg. The agar was dissolved in 100°C. distilled water, brass-wool was added and air lead through the solution for 20 minutes at 70°C. The brass-wool was subsequently washed with alcohol and ether and placed in a small test tube, the upper end of which was melted to form a capillary, the tube was heated to a glow and Hg sublimated in the cooler part of the tube. Further control was made as follows: the capillary was cut off and placed in a larger test tube, at the bottom of which were put iodine crystals. At heating the colour of the sublimated Hg changed to red.

As. The ash was dissolved in diluted H_2SO_4 in a test tube, a piece of Zn, free from As, was added. Cotton was inserted as a filter, in the upper end of the tube, the opening of which was covered with a piece of filterpaper, upon which was placed a piece of pure silver

nitrate. The tube was slightly heated, after a few minutes the colour of the silver nitrate changed to yellow and soon thereafter to black. (Method of *Gutzeit*)

Sb. The ash was dissolved in HCl, whereafter the metal was precipitated with H_2S .

Bi was shown by the same method.

Te. The ash was dissolved in HCl, weak precipitation took place with H_2S . A sample taken from the precipitate coloured the gas flame in the characteristic way.

When the atomic weight of elements is high, Roentgen methods may be successfully applied in order to show the existence of elements in the culture media. During these experiments Roentgen

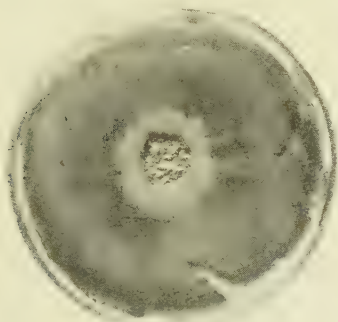


Figure VII.

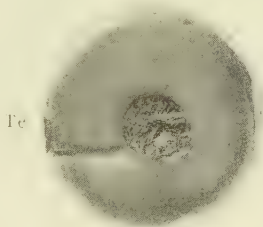


Figure VIII.

photography was used to show the existence of Cu, Co, Zn and Tl. When taking Roentgen pictures of elements possibly spread in the agar it is not always suitable to use ordinary petri dishes, because the glass itself may contain substances of high atomic weight which would absorb the X-rays and thus conceal the small quantities of the matter under investigation. For this purpose were made special dishes of 4 per cent collodium elasticum, as illustrated in picture XV. Soon it was found, however, that such dishes could only be used for substances which become quickly spread in the culture media, because the water forming part of the latter dialyzes comparatively quickly into the surroundings. Later on the following method was adopted: a Roentgen film was stripped of its light sensitive layers and was cut into round pieces fitting into a petri dish, a series of slips or «cogs» were left around the periphery of these pieces of film to be bent upwards and cut level with the rim of the dish; this was done in

order to enable the film to be pressed down to the bottom of the dish after the agar had been poured into it. When photographing, this loose bottom supporting the agar may be lifted up, as illustrated in figure XVI.

Figure XVII shows an X-ray picture of Cu taken in the way described above. It represents the same dish as in picture V, but the Cu piece is removed. The light green border round the metal produces a distinct shadow in the film and a spectroscopic Cu colour. Thus, in this case, Cu has become disengaged from the metal and its presence in the culture media can be demonstrated. It is of interest to note that the metal, by this method, can be found in the agar only, along

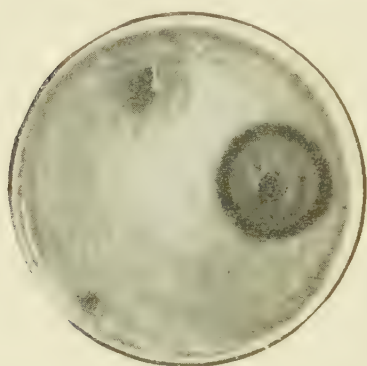


Figure IX.



Figure X.

the margin of the Cu piece. Figure XVIII is an X-ray picture of a dish with Co. The metal is placed on the agar, arrows point to a line, where a thin collodion partition has been inserted; to the left of the latter the agar is clearly red, to the right of it the agar has its ordinary colour. The X-ray picture shows that the left side of the dish is somewhat darker than the right one, this is best seen in the place where the partition is. This blackening is evidently due to the presence of Co in the agar.

As already mentioned above, Zn and Tl were only used to demonstrate their process of spreading in the culture media. Figure XIX, which is an X-ray picture of XIII, shows at the point of Zn 1 a blackening of the film which corresponds with the coagulation, visible in figure XIII. At Zn 2 the film appears unblackened round Zn. Figure XX is an X-ray picture of XIV. The latter shows white spots which absorb the X-rays and produce a shadow.



Figure XI.

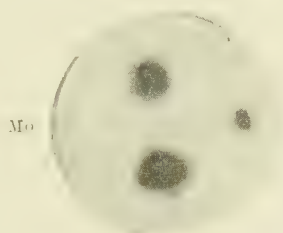


Figure XII.

Said spots grow smaller the farther they are from the element. Owing to the high atomic weight of Tl, 204,39, even the smallest particles are clearly visible.

The tests referred to above have disclosed 9 out of 10 elements with which experiments were carried out in the culture media. Zn and Tl were also proved. As already mentioned, direct contact between the element and the culture media is a necessity and, according to my opinion, an inevitable condition for demonstrating the oligodynamic action of elements in agar cultures. Bacteria free zones, or such without any visible growth, are doubtlessly caused by elements which have spread in the culture media, although the bacteria in this region are still able to retain their divisibility for quite a long time. I do not find anything to support the theory that the effect produced by elements should be due to radiation.

Under the influence of the elements some bacteria lost their pigment, e. g. *B. prodigiosum* and *Pseudomonas pyocyanea*. It is here a question of such colonies which developed on new culture media after having been transplanted from the bacteria free zone. As

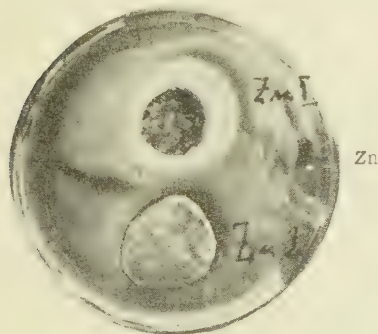


Figure XIII.

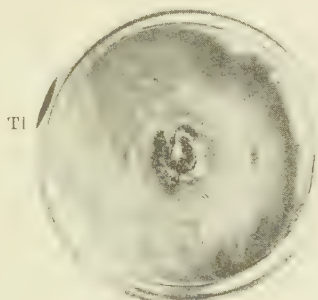


Figure XIV.

soon as the colonies changed their colour they were used to produce new cultures in order to ascertain during what period the loss of pigment could be observed.

B. prodigiosum. Cu caused no constant discoloration, the bacteria died fairly quickly under the influence of this metal. With Ag discoloration began on the 4th day, but did not prove constant; colonies, transplanted as late as on the 7th day, were slightly coloured round

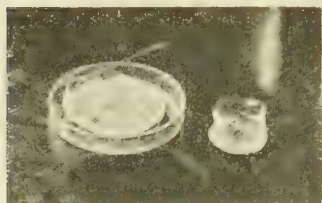


Figure XV.

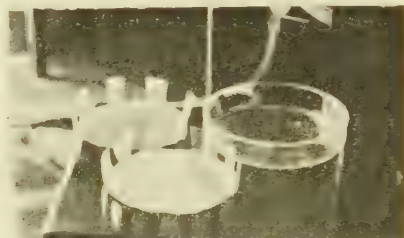


Figure XVI.



Figure XVII.



Figure XVIII.

the metal and recovered their red dye. With Au the loss of pigment began on the 5th day and from the 10th day this process of discolouring became constant and was observed through 20 generations. Cd caused discoloration on the 4th day and from the 9th day on no red dye could be observed through 20 generations. Hg failed to discolour the bacteria. As destroyed the colonies in 5 days; the discolouring process began after 3 days, but in a fresh culture the bacteria recovered their colour. Sb began to discolour the bacteria on the 3rd day and after 8 days the light dye became constant for 20 generations. Bi

caused no constant discoloration, from the 10th day on the colonies were grey, but already in the following generation the usual dye was restored. Te was partly effective on the 9th day, but the red colour returned in the following generation. Co did not produce any discoloration. With *B. prodigiosum* a discoloration which lasted fairly long, a pigment-free bacteria, was obtained by use of the elements Au, Cd and Sb.

Pseudomonas pyocyanea. It was observed that the colonies of this bacteria lost their usual colour after having been in touch with the elements for some time; they became light of colour and the dye of the culture media failed to develop. Cu and Ag destroyed

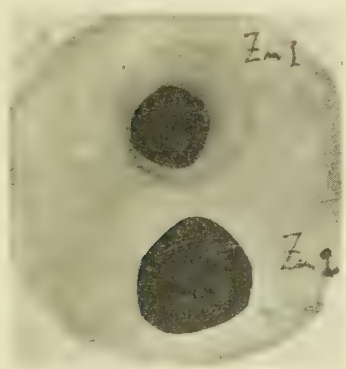


Figure XIX.

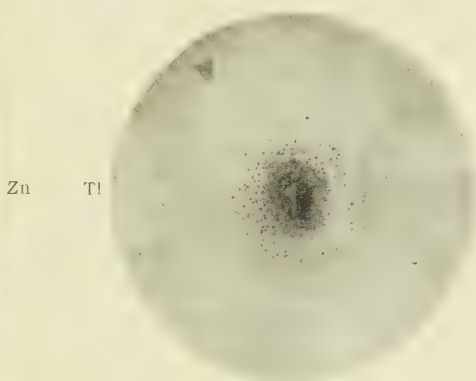


Figure XX.

the bacteria comparatively quickly; it was therefore impossible to produce more than a few light cultures which were observed to recuperate their colour in the following generation. Au produced after 9 days constant discoloration which was observed through 20 generations. Exactly such was the case also with Cd. Hg did not cause any constant discoloration of the bacteria. This can also be said of As, the bacteria died after having shown light coloured colonies for 3 to 5 days. Sb caused an inconstant discoloration from the 3rd day and from the 8th day the discoloration became constant through 20 generations. Bi did not discolour the new cultures. Te produced a pigment-free bacteria on the 7th day, but any constant discoloration did not take place, the bacteria lived 9 days with this metal.

These experiments show that certain elements, notably Au, Cd and Sb, when allowed sufficient time to influence *Pseudomonas pyocyanea*, paralyze the latter's capacity to produce a dye. Parallel with this the culture media remains undyed.

CHAPTER 11.

Conclusions.

The present treatise has served to ascertain the following: Previous statements of a great number of scientists to the effect, that bacteria in the zone nearest the element, the so-called bacteria free region, were actually dead, does not hold good, as even this region contains bacteria, which, however, cannot divide, owing to the deleterious effect of elements, but are nevertheless a considerable time able to live and grow when transplanted to a fresh culture media. Murro's opinion is herewith confirmed.

There exists a certain conformity between the size of the bacteria free region and the life length of the bacteria. A larger bacteria free region usually means a quicker destruction of bacteria. Of all the elements referred to in this paper, Cd, Hg, As and Sb proved to have the most powerful germicidal action, this may be said also about Cu and Te in quite a number of cases. On the other hand Au and Bi showed themselves to be exceedingly weak in this respect, Ag and Co were slightly more effective.

It may be assumed also with some certainty that the agar as a protective colloid prevents to a varying extent the full development of the powers characteristic to the different elements.

During the experiments referred to above Mycobacteria were the easiest to affect and they also showed a larger bacteria free region than the others. In the majority of cases the death of bacteria is preceded by a weakening and diminishing of colonies.

The best effect is obtained by allowing the elements to rest on the agar, in direct contact with it. The oligodynamic effect of different elements chiefly depends upon their spreading in the culture media.

With the exception of Au all the elements used could be shown to exist in the culture media; in the case of Cu and Co this was proved by X-rays. Zn and Tl were also traced in the same way, but these elements were not used in the real experiments.

Bacteria which form pigment can lose this property after lasting exposure to direct contact of different elements.

References.

- AGULHON, H. et mille ROBERT, TH.: Action de l'uranium colloïdal sur le bacille pyocyanique. C. R. Acad. Sciences. 1914, T. 158, p. 439.
- et SAZERAC, R.: Action des sels d'uranium et de l'uranium métallique sur le bacille pyocyanique. C. R. Acad. Sciences. 1913, T. 156, p. 162.
- ANDRESEN, P. H.: Über den Einfluss von Metallsalzen auf die Entwicklung der Bakterien. I. Silbersalze. Zbl. Bakter. 1927/28, I, Orig. Bd. 105, p. 444.
- : Über den Einfluss von Metallsalzen auf die Entwicklung der Bakterien. II. Silbersalze. Zbl. Bakter. 1928, I, Orig. Bd. 107, p. 392.
- V. ANGERER, K.: Untersuchungen an Wasserspirochäten. Archiv. f. Hygiene. 1922, Bd. 91, p. 201.
- ANJOW, SABURO: Vergleichende Untersuchungen über die Widerstandsfähigkeit verschiedener Leptospiren (Spirochäten) gegen äussere Einflüsse. Zbl. Bakter. 1928, I, Orig. Bd. 109, p. 61.
- BAIL, O.: Über das Verhalten Gram-positiver und -negativer Bakterien zu oligodynamischen Wirkungen. Wien. klin. Wschr. 1919, 29, p. 751.
- BAUMGARTEN, A. und LUGER, A.: Zur Theorie des sogenannten oligodynamischen Phänomens. (Erwiderung auf die Bemerkungen Paul Saxls). Wien. klin. Wschr. 1918, 7, p. 188.
- BECHOLD, H. und ZIEGLER, J.: Die Beeinflussbarkeit der Diffusion in Gallerten. Zeitschr. f. physikal. Chemie. 1906, Bd. 56, p. 105.
- BECKER, A.: Physik der radioaktiven Messmethoden. Lehrbuch der Strahlentherapie. 1925, Bd. 1.
- BECQUEREL, P.: Influence des sels d'uranium et de thorium sur le développement du bacille de la tuberculose. C. R. Acad. Sciences. 1913, T. 156, p. 164.
- BEGGER, H.: Über aktive Immunisierung mit »gekupferten« Spirochätenkulturen bei der Weilschen Krankheit. Zbl. Bakter. 1924, I, Orig. Bd. 91, p. 90.
- BEHRING: Über Desinfektion, Desinfektionsmittel und Desinfektionsmethoden. Zeitschr. f. Hygiene. 1890, Bd. 9, p. 395.
- BERTRAND, GABRIEL: Sur le rôle capital du manganèse dans la formation des conidies de l'*Aspergillus niger*. C. R. Acad. Sciences. 1912, T. 154, p. 381.
- : Extraordinaire sensibilité de l'*Aspergillus niger* vis à-vis du manganèse. C. R. Acad. Sciences. 1912, T. 154, p. 616.
- : L'argent peut-il, à une concentration convenable, exciter la croissance de l'*Aspergillus niger*. C. R. Acad. Sciences. 1914, T. 158, p. 1213.
- et JAVILLIER, M.: Action du manganèse sur le développement de l'*Aspergillus niger*. Annal. de l'Inst. Pasteur 1912, T. 26, p. 241.
- et JAVILLIER, M.: Action combinée du manganèse et du zinc sur le développement et la composition minérale de l'*Aspergillus niger*. Annal. de l'Inst. Pasteur. 1912, T. 26, p. 515.

- BIASIOTTI, A.: L'azione dei metalli colloidali sui germi patogeni. (Ann. d'Igiene speriment. 1909, Vol. XIX, Fasc. 4, p. 543). Ref: Zbl. Bakter. 1910, I, Ref. Bd. 45, p. 680.
- BOKORNY, TH.: Quantitative Wirkung der Gifte. Pflügers Archiv. 1906, Bd. 111 p. 341.
- BORNARD, M.: Influence des métaux sur le développement de l'*Aspergillus niger* cultivé sur un liquide de Raulin. Zbl. Bakter. 1913, II, Bd. 39, Nr. 18—19. Ref: Bull. de l'inst. Pasteur. 1914, 5, p. 212.
- BRUNNER, OTTO: Über die Beziehungen der chemischen Konstitution zur pharmakologischen Wirkung bei Antimonpräparaten. Archiv f. exp. Pathol. und Pharmakol. 1912, Bd. 68, p. 186.
- BRUNTZ, L. et SPILLMAN, L.: Sur le mécanisme de l'action thérapeutique des injections des métaux colloïdaux. C. R. Soc. Biol. 1911, T. 70, p. 298.
- BUCHSTAB, L.: Zur Frage über die oligodynamischen Wirkungen. Odesskij medicinsk'j Žurnal. Jg. 3, Nr. 2, p. 57, 1928. (Russisch). Ref: Zbl. f. d. ges. Hygiene. 1929, Bd. 18, p. 662.
- BÜRGI, E. und LAUBENHEIMER, K.: Desinfektion und Sterilisationslehre. 2. Chemischer Teil. Handb. d. pathogen. Mikroorg. v. Kolle, Kraus und Uhlenhuth. 1931, Bd. III, Teil II, p. 987.
- BUSCHKE, A. und JAKOBSON, F.: Untersuchungen über Thalliumwirkung. Dtsch. med. Wschr. 1922, 26, p. 859.
- — — und JAKOBSON, F. und KLOPSTOCK, E.: Über das Wesen der oligodynamischen antibakteriellen Metallwirkung. Dtsch. med. Wschr. 1925, 15, p. 595.
- LA CAVA, G.: Azioni oligodinamiche dei metalli sui batteri. Ann. Igiene. 1931, 41, 612—619. Ref: Zbl. Bakter. 1932, I, Ref. Bd. 105, p. 430.
- CERNOVODEANU, P. et HENRI, VICTOR: Action de l'argent colloïdal sur quelques microbes pathogènes. Importance du mode de préparation et de la grosse des granules du colloïde. C. R. Soc. Biol. 1906, T. 60, p. 122.
- — — et STODEL, G.: Action du mercure colloïdal électrique sur quelques microbes pathogènes. C. R. Soc. Biol. 1908, T. 64, p. 1063.
- CHARRIN et PHISALIX: Abolition persistante de la fonction chromogène du bacillus pyocyaneus. C. R. Acad. Sciences. 1892, T. 114, p. 1565.
- CLARK, H. W. and GAGE, S. D.: On the bactericidal action of the copper. Journ. of Inf. Dis. Suppl. Nr. 2, p. 175. Ref.: Zbl. Bakter. 1908, I, Ref. Bd. 41, p. 191.
- CLUZET, ROCHAIX et KOFFMAN: Action bactéricide du radium sur le bacille pyocyanique. C. R. Soc. Biol. 1920, T. 83, p. 1043.
- — — — — : Action bactéricide du radium sur le bacille pyocyanique, C. R. Soc. Biol. 1920, T. 83, p. 1428.
- — — — — : Action bactéricide du radium sur le B. d'Eberth. Variations de la dose bactéricide. C. R. Soc. Biol. 1920, T. 84, p. 37.
- COBER, R. und van der REIS V.: Über den Einfluss der arsenigen Säure auf das Bakterienwachstum. Biochem. Zeitschr. 1922, Bd. 129, p. 73.
- COLLIER, W. H.: Versuche über den Einfluss der Bestrahlung auf Trypanosomen und Bakterien. Zeitschr. f. Hygiene und Infektionskr. 1931, Bd. 112, p. 724.
- COOPER, E. A. und NICHOLAS, S. D.: Bakteriologische Chemie der Schwermetallen. Soc. chem. Ind. 1930, 49, p. 386. Ref: Zbl. Bakter. 1931, I, Ref. Bd. 104, p. 92.

- DOERR, R.: Zur Oligodynamie des Silbers. Biochem. Zeitschr. 1920, Bd. 106, p. 110.
- »— Zur Oligodynamie des Silbers. II Mitteilung. Biochem. Zeitschr. 1920, Bd. 107, p. 207.
- »— Zur Oligodynamie des Silbers. III Mitteilung. Biochem. Zeitschr. 1921, Bd. 113, p. 58.
- »— und BERGER, W.: Zur Oligodynamie des Silbers. IV Mitteilung. Biochem. Zeitschr. 1922, Bd. 131, p. 351.
- EGGERTH, ARNOLD, H.: Changes in the stability and potential of cell suspensions. I. The stability and potential of *Bacterium coli*. Journ. of Gen. Physiol. 1924, VI. p. 63.
- EICHBAUM, FRANZ: Die Wertbestimmung »oligodynamischer« Silberwässer im biologischen Versuch. Zbl. Bakter. 1932, I. Orig. Bd. 126, p. 128.
- ELFVING, FR.: Über physiologische Fernwirkung einiger Körper. Commentationes variae in memoriam actorum CCL annorum. Edidit Universitas Helsingforsiensis, 1890.
- »—: Zur Kenntnis der pflanzlichen Irritabilität. Öfversigt af Finska Vetenskaps-societetens Förhandlingar. 1893—94, Bd. XXXVI, p. 77.
- »—: Phycomyces und die sogenannte physiologische Fernwirkung. Översikt av Finska Vetenskaps-societetens Förhandlingar. 1916—17, Bd. LIX, Avd. A, Nr 18.
- FALTA, W. und RICHTER-QUITTNER, M.: Über die sogenannte oligodynamische Wirkung von Schwermetallen und Schwermetallsalzen. Biochem. Zeitschr. 1921, Bd. 115, p. 39.
- FICKER, M.: Über Lebensdauer und Absterben von pathogenen Keimen. Zeitschr. f. Hygiene und Infektionskr. 1898, Bd. 29, p. 1.
- FISCHER, M.: Über das Wesen der Oligodynamie und ähnlicher Reizerscheinungen. (Vorläufige Mitteilung). Archiv. f. Hygiene. 1924, Bd. 94, p. 214.
- FISCHL, V.: Periodisches System und Chemotherapie. Z. angew. Chemie, 1931, 44, p. 932. Ref: Zbl. Bakter. 1932, I, Ref. Bd. 105, p. 419.
- FOÁ, CARLO und AGGAZZOTTI, ALBERTO: Über die physiologische Wirkung kolloidaler Metalle. Biochem. Zeitschr. 1909, Bd. 19, p. 1.
- »— —»— Dell' azione battericida e antitossica di alcuni metalli colloidal. R. Clin. Med. di Torino. 1907. Maggio. Ref: Zbl. Bakter. 1908, I, Ref. Bd. 41, p. 189.
- FREUNDLICH, HERBERT: Kolloidchemie und Biologie. Dresden 1924.
- FREUNDLICH, H. und SÖLLNER, K.: Zur Erklärung der oligodynamischen Wirkung. Biochem. Zeitschr. 1928, Bd. 203, p. 1.
- FRIEDBERGER, E. und JOACHIMOGLU, G.: Über die Anhängigkeit der keimtötenden und entwicklungshemmenden Wirkung von der Valenz. Biochem. Zeitschr. 1917, Bd. 79, p. 135.
- FRIEDENTHAL, HANS: Absolute und relative Desinfektionskraft von Elementen und chemischen Verbindungen. Biochem. Zeitschr. 1919, Bd. 94, p. 47.
- FRIEDMANN, ERNST A. H.: Pyocyanine, an accessory respiratory ferment. Journ. of. exp. Medicine. 1931, Vol. 54, No 2, p. 207.
- FROUIN, A. et MERCIER, V.: Action du vanadate de soude sur le développement de l'*Aspergillus*. Bull. Soc. Chim. biol. 1914, T. I. p. 8. Ref: Bull. de l'Inst. Pasteur. 1914, 22, p. 890.

- GOTTSCHALK, H.: Eine einfache Standardmethode für oligodynamische Versuche mit Silber. Zbl. Bakter. 1931, I, Orig. Bd. 122, p. 400.
- : Über die Beeinflussung der oligodynamischen Wirkung. Zbl. Bakter. 1932, I, Orig. Bd. 123, p. 468.
- GUTSTEIN, H.: Über die Giftigkeit von Schwermetallsalzen für Mikroorganismen. Zbl. Bakter. 1932, I, Orig. Bd. 124, p. 572.
- HAAAS, ARTHUR: Atomtheorie. 1929.
- HÄMÄLÄINEN, R.: Über die oligodynamische Einwirkung der Silbersalze auf den *Staphylococcus aureus*. Acta Soc. Medic. Fenn. Duodecim. 1930, 13, Fasc. 2.
- HERZBERG, KURT: Die Beteiligung des Sauerstoffs bei der oligodynamischen Metallwirkung. I Mitteilung. Zbl. Bakter. 1923, I, Orig. Bd. 90, p. 113.
- : Die Beziehung des Sauerstoffs zur oligodynamischen Metallwirkung. Zbl. Bakter. 1923, I, Ref. Bd. 74, p. 489.
- HEYDWEILLER, A. und KOPFERMANN, F.: Zur Kenntnis der Glaselektrolyse. Annal. d. Physik. 1910, Bd. 32, p. 739.
- HIRSCH, KURT: Die Kupferresistenz der Spirochäten vom Weil-Typ und Versuche über die Reinzüchtung von Wasserspirochäten auf Kupfernährböden. Zeitschr. f. Immun. forschg. 1931, Bd. 71, p. 459.
- HODER, F.: Über Oligodynamie. Münch. med. Wschr. 1932, 46, p. 1828.
- HOEMANN, PAUL: Über die Gültigkeit des Arndt-Schulzischen biologischen Grundgesetzes bei der Wirkung von Bakteriengiften. Archiv f. Hygiene. 1922, Bd. 91, p. 231.
- : Studien über die oligodynamische Wirkung von Metallen und Metallsalzen auf Bakterien bei verschiedenen Sauerstoffspannungen. Zbl. Bakter. 1929, I, Orig. Bd. 114, p. 216.
- HORELLI, VÄINÖ: Über die Oligodynamie bei Mykobakterien. Mit besonderer Berücksichtigung der Theorie von J. A. Murto. Acta Soc. Medic. Fenn. Duodecim. 1930, 13, Fasc. 2.
- HÜNE: Die begünstigende Reizwirkung kleinster Mengen von Bakteriengiften auf die Bakterienvermehrung. Zbl. Bakter. 1909, I, Orig. Bd. 48, p. 135.
- ISRAEL, O. und KLINGMANN, Th.: Biologische Studien mit Rücksicht auf die Pathologie. III. Oligodynamische Erscheinungen (v. Nägeli) an pflanzlichen und tierischen Zellen. Virchows Archiv. 1897, Bd. 147, p. 293.
- JAVILLIER, M.: Sur la substitution au zinc de divers éléments chimiques pour la culture du *Sterigmatocystis nigra*. C. R. Acad. Sciences. 1912, T. 155, p. 1551.
- : Une cause d'erreur dans l'étude de l'action biologique des éléments chimiques: la présence de traces de zinc dans le verre. C. R. Acad. Sciences. 1914, T. 158, p. 140.
- : Sur la culture de l'*Aspergillus Niger* (*Sterigmatocystis nigra* V. Tgh) dans le milieu où le zinc est remplacé par divers éléments chimiques (cuivre, uranium, vanadium). Bull. Soc. Chim. biol. 1914, T. 1, p. 55. Ref: Bull. de l'Inst. Pasteur. 1914, 22, p. 890.
- et m^{me} TSCHERNOROUTZKY, H.: Influence comparée du zinc, du cadmium et du glucinium sur la croissance de quelques Hyphomycetes. C. R. Acad. Sciences. 1913, T. 157, p. 1173.
- KARCZAG, L.: Strahlung und Kolloide. Handb. d. ges. Strahlenheilk., Biologie, Pathologie und Therapie. 1928, Bd. 1, p. 403.

- KONRICH, F.: Über oligodynamische Trinkwassersterilisierung mittels des, Katodyn-Verfahrens. *Gesdh. ing.* 1929, 52, p. 804. Ref: Zbl. Bakter. 1931, I, Ref. Bd. 99, p. 334.
- V. KORANYI und RICHTER, P. F.: *Physikalische Chemie und Medizin*, Leipzig 1907.
- KRAMERS, H. A. und HOLST, HELGE: *Das Atom und die Bohrsche Theorie seines Baues*. Berlin 1925.
- KRUYT, H. R.: *Einführung in die physikalische und Kolloidchemie*. Leipzig 1926.
- KRÖNIG, B. und PAUL, Th.: *Die chemischen Grundlagen der Lehre von der Giftwirkung und Desinfektion*. *Zeitschr. f. Hygiene und Infektionskr.* 1897, Bd. 25, p. 1.
- KUROKAWA, A.: Über die hämolytische und baktericide Wirkung verschiedener Arten von Metallpulvern. *Zeitschr. f. Immun. forschg.* 1925, Bd. 44, p. 127.
- LAGRANGE, E.: Action à distance des métaux sur les colibacilles. *C. R. Soc. Biol.* 1932, T. 109, 4—5. Ref: Zbl. Bakter. 1932, I, Ref. Bd. 106, p. 409.
- LAPPALAINEN, HANNA: *Biochemische Studien an Aspergillus niger*. Akad. Abdh. Helsingfors 1919.
- LASAGNA, F.: Contributo sperimentale e clinico sulla azione antibatterica e terapeutica dei metalli colloidali. *Rif. Med.* 1907. Nr 39. Ref: Zbl. Bakter. 1910, I, Ref. Bd. 41, p. 189.
- LASSEUR, P., PIERRET, M., DUPAIX, A. et MAGNITOT, C.: Remarques sur le pouvoir bactéricide de l'argent métallique. *C. R. Acad. Sciences.* 1932, T. 194, p. 1024. Ref: Zbl. Bakter. 1932, I, Ref. Bd. 107, p. 526.
- LAUBENHEIMER, K.: Über die Einwirkung von Metallen und Metallsalzen auf Bakterien und Bakteriengifte. Versuche zur praktischen Verwertung der oligodynamischen Wirkung von Metallen. *Zeitschr. f. Hygiene und Infektionskr.* 1921, Bd. 92, p. 78.
- LEHMANN, K. B. und NEUMANN, R. O.: *Bakteriologie und Bakteriologische Diagnostik*. 6 Auflage.
- LEITNER, N.: Zur Phänomenologie der oligodynamischen Wirkung. Die Salzhemmung. *Zbl. Bakter.* 1929, I, Orig. Bd. 112, p. 368.
- : Der Einfluss von Elektrolyten auf die baktericide Wirkung von Kupfer- und Silbersalzen. Die Abhängigkeit der bakteriziden Wirkung von der elektrostatischen Ladung der Bakterien. (Erklärung der sogenannten Salzhemmung der oligodynamischen Wirkung). *Biochem. Zeitschr.* 1930, Bd. 221, p. 42.
- LEPIERRE, CH.: Remplacement du zinc par l'uranium dans la culture de l'*Aspergillus niger*. *C. R. Acad. Sciences.* 1913, T. 157, p. 1179.
- LIESEGANG, Ed. H.: *Beiträge zu einer Kolloidchemie des Lebens*. Biologische Diffusionen. Dritte umgearb. Auflage. 1923.
- LINDEN, M., GRÄFIN VON: Die entwicklungshemmende Wirkung von Kupfersalzen auf Krankheit erregende Bakterien. *Zbl. Bakter.* 1921, I, Orig. Bd. 85, p. 136.
- : Entwicklungshemmende Wirkung von Kupfer-Glasverbindungen auf das Wachstum von Bakterien. *Zbl. Bakter.* 1922, I, Orig. Bd. 87, p. 310.
- LOUROS, N. und SCHEYER, H. E.: *Stafylokokkeninfektionen, das Retikuloendotelialsystem, ihre Beziehungen und ihre therapeutische Beeinflussbarkeit*. VI Mitteilung. Therapeutische Versuche mit Metalle und Metallsalze. *Zeitschr. f. d. ges. exp. Medizin.* 1927, Bd. 57, p. 22.

- LÖHNER, L.: Über »keimfreie Höfe« und »Randwulstbildungen« als biologische Folgen oligodynamischer Metallwirkungen. Wien. klin. Wschr. 1919, 37, p. 911.
- und MARKOVITS, B. E.: Zur Kenntnis der oligodynamischen Metall-Giftwirkungen auf die lebendige Substanz. I Mitteilung: Paramäcienversuche. Pflügers Archiv. 1922, Bd. 195, p. 417.
- LUGER, A.: Über die durch Metalle, Metallsalze und flüchtige Desinficientien hervorgerufenen keimfreien Höfe auf Bakterienplatten. Wien klin. Wschr. 1920, 38, p. 833.
- LUSINI, V.: Azione disinfettante dei cationi secondo la legge del sistema periodico. R. Accademia dei Fisiocritici. Sitz. v. 20 März 1910. Ref: Gazz. Osped. e clin., 1910, 40 p. 431. Ref: Zbl. Bakter. 1910, I, Ref. Bd. 47, p. 610.
- MARBOE, FRANZ: Über den Einfluss blanker Metalle auf Hefe. Zbl. Bakter. 1930, II, Bd. 81, p. 67. Ref: Zbl. Bakter. 1931, I, Ref. Bd. 101, p. 422.
- MESSERSCHMIDT, TH.: Das Desinfektionsvermögen der Metalle und seine Ursachen mit besonderer Berücksichtigung der Wirkung des Kupfers. Zeitschr. f. Hygiene und Infektionskr. 1916, Bd. 82, p. 289.
- Über keimtötende Eigenschaften von Geschossen. Med. Klin. 1916, 14, p. 444.
- MILLER, W.: Über die antiseptische Eigenschaft einiger Goldpräparate. Verhandlungen der deutschen odontologischen Gesellschaft. 1889, Bd. 1, p. 100.
- MITTELBACH, H.: Über die desinfizierende Wirkung der Kupfersalze. Zbl. Bakter. 1921, I, Orig. Bd. 86, p. 44.
- MUNN, LOTTIE E. and HOPKINS, B. S.: Studies on tellurium. The value of some tellurium compounds as disinfectants. Journ. of Bacteriol. 1925, X, p. 79.
- MURTO, J. A.: Über die Oligodynamie. Acta Soc. Medic. Fenn. Duodecim. 1930, 13, Fasc. 2.
- Über die Infektionsimmunität. Sitzungsber. d. finn. Akademie d. Wissenschaften. 1930, p. 49.
- V. NÄGELI, CARL: Über oligodynamische Erscheinungen in lebenden Zellen. Mit einem Vorwort von S. Schwendener und einem Nachtrag von C. Cramer. Separatabdruck aus den Denkschriften der schweizerischen naturforschenden Gesellschaft. Bd. XXXIII, 1, 1893.
- V. NEERGAARD, K.: Experimentelles zur intravenösen Silbertherapie. V Mitteilung. Spielen unbekannte physikalische Kräfte im Sinne der Oligodynamie Sachs eine Rolle bei der intravenösen Silbertherapie? Archiv f. exp. Pathol. und Pharmacol. 1925, Bd. 109, p. 164.
- NĚMEC, ANTONIN und VÁCLAV KÁŠ: Über den Einfluss des Selens auf die Entwicklung einiger Schimmelpilze aus der Gattung *Penicillium*. Biochem. Zeitschr. 1920, Bd. 111, p. 12.
- NORTHROP, J. H. and de KRUIF, PAUL H.: The stability of bacterial suspensions. II. The agglutination of the bacillus of rabbit septicaemia and of bacillus typhosus by electrolytes. Journ. of Gen. Physiol. 1921—22. IV, p. 639. Referred to by Leitner.
- OSTWALD, WO.: Die Welt der vernachlässigten Dimensionen. Neunte und zehnte Auflage. 1927.

- PAULI, WO. und FLECKER, LEO: Untersuchungen über physikalische Zustandsänderungen der Kolloide. XIII. Beziehungen von Eiweiss zu anorganischen Kolloiden und Schwermetallsalzen. *Biochem. Zeitschr.* 1912, Bd. 41, p. 461.
- PFAB, BRUNO: Über die oligodynamische Wirkung der Metalle. *Münch. med. Wschr.* 1928, 45, p. 1917.
- : Über Verbrennungen und ihre Behandlung mit Silber. *Münch. med. Wschr.* 1930, 20, p. 857.
- PILOD et CODVELLE: Action du cuivre métallique sur les germes des eaux d'alimentation. *C. R. Acad. Sciences.* 1932, T. 194, p. 497. *Ref. Zbl. Bakter.* 1932, I, *Ref. Bd.* 107, p. 518.
- PIORKOWSKI, SUSE: Untersuchungen über die keimtötende (oligodynamische) Wirkung des in der Zahnheilkunde verwandten Wurzelfüllmaterials aus chemisch reinem Silber. *Zahnärztl. Rundschau.* 1932, 6, p. 224 und 7, p. 266.
- PITFIELD, ROBERT L.: *Compend of Bacteriology.* Fourth Edition. Philadelphia 1922.
- PÖSCHL, VIKTOR: Einführung in die Kolloidchemie. Sechste verb. Aufl. Dresden 1923.
- RANKIN, ALLAN C.: The germicidal action of metals and its relation to the production of peroxide of hydrogen. *Proc. Royal Soc. Ser.* 1910, B. Vol. 82, p. 78.
- ROBERT, MILLE: Influence du calcium sur le développement et la composition minérale de l'*Aspergillus niger*. *C. R. Acad. Sciences.* 1911, T. 153, p. 1175.
- ROOSEN, RUDOLF: Zur Metalltherapie des Typhus abdominalis und der septischen Allgemeininfektionen. *Schw. med. Wschr.* 1930, 40 p, 951.
- ROSENKRANZ, H.: Untersuchungen über die praktische Verwertbarkeit der oligodynamischen Wirkung der Kupfersalze auf Bakterien. *Archiv f. Hygiene* 1920, Bd. 89, p. 253.
- RIED, O.: Biologische Wirkung photechischer Substanzen. I Mitteilung. Versuche über biologische Wirkung photechisch wirksamer Metalle. *Wien. klin. Wschr.* 1930, 13, p. 394.
- : Biologische Wirkung photechischer Substanzen. II Mitteilung. Versuche über die Wirkung bestrahlter Metalle auf Bakterien. *Zbl. Bakter.* 1931, I, *Orig. Bd.* 121, p. 267.
- RISLER, J.: Action bactéricide de l'argent irradié. *La presse Méd.* 1929, p. 1430.
- RUETE, A. E.: Über die oligodynamische Wirkung unseres Silberpulvers. *Klin. Wschr.* 1925, 52, p. 2499.
- SALUS, G.: Oligodynamische Metallwirkungen. *Wien. klin. Wschr.* 1919, 51, p. 1220.
- SAUTON, B.: Le fer est-il indispensable à la formation des spores de l'*Aspergillus niger*? *C. R. Soc. Biol.* 1911, T. 71, p. 589.
- SAXL, PAUL: Die oligodynamische Wirkung der Metalle und Metallsalze. *Wien* 1924.
- SCHLOSSBERGER, H.: Über die keimtötende Wirkung der Metalle und Metallsalze. *Med. Klin.* 1918, 9, p. 204.
- SCHNABEL, A.: Über Metallwirkung auf Bakterien. *Klin. Wschr.* 1922, 8, p. 389.
- SCHUHMACHER, J.: Über oligodynamische Metallwirkungen. *Biochem. Zeitschr.* 1922, Bd. 134, p. 398.

- SCHULTZ, GÜNTHER: Versuche über die Diffusion von Silber in Glas. *Annal. d. Physik.* 1913, Bd. 40, p. 335.
- SCHWARTZ, W. und STEINHART, H.: Untersuchungen über die oligodynamische Wirkung des Kupfers. I Teil. *Archiv f. Mikrobiol.* 1931, 2, p. 261. Ref: *Zbl. Bakter.* 1931, I, Ref. Bd. 103, p. 239.
- SEIFFERT, W.: Untersuchungen über den Einfluss oligodynamischer Metallwirkungen auf das Wachstum von Bakterien. (Ein Beitrag zu Arndts biologischem Grundgesetz). *Münch. med. Wschr.* 1920, 50, p. 1437.
- : Der Löhnersche Randwulst am keimfreien Hof als Stütze des Arndtschen Grundgesetzes. *Biochem. Zeitschr.* 1922, Bd. 129, p. 50.
- SHIGA, AKIRA: Untersuchungen über die Beziehungen der Wasserspirochäte (*Spirochaeta pseudoicterogenes* Uhlenhuth u. Zuelzer) zu dem Erreger der Welschen Krankheit (*Spirochaeta icterogenes* Uhlenhuth u. Fromme s. *Spirochaeta icterohaemorrhagiae* Inada u. Ido). *Zeitschr. f. Immunforsch.* 1924, Bd. 40, p. 148.
- SMITH, ALEXANDER und D'ANS, J.: Einführung in die allgemeine und anorganische Chemie. VI Aufl. 1931.
- SORDELLI, A. et WERNICKE, R.: Recherches sur l'oligodynamie. Activation de l'eau par le cuivre. *C. R. Soc. Biol.* 1921, T. 85, p. 317.
- SPÄT, W.: Untersuchungen über oligodynamische Fernwirkungen. *Wien. klin. Wschr.* 1920, 24, p. 509.
- SPIRO, K.: Die oligodynamische Wirkung des Kupfers. *Münch. med. Wschr.* 1915, 47, p. 1601.
- : Die oligodynamische Wirkung des Kupfers. Ein Beitrag zur Lehre vom Antagonismus. *Biochem. Zeitschr.* 1916, Bd. 74, p. 265.
- SPRINGER, A. and SPRINGER, A., J.R.: Antiseptic action of copper. *Journ. of ind. and engin. Chem.* 1901, p. 676. Ref: *Zbl. Bakter.* 1911, I, Ref. Bd. 48, p. 563.
- STOKLASA, JULIUS und PĚNKAVA, JOS.: Biologie des Uraniums. *Biochem. Zeitschr.* 1928, Bd. 194, p. 15.
- STRECK, A.: Über die oligodynamische Wirkung des Kupfers auf Bakterien. *Hyg. Rundschau.* 1919, Jg. 29, Nr 20, p. 687; Nr 21, p. 717.
- SÜPFLE, K.: Über die oligodynamische Metallwirkung auf Bakterien. *Münch. med. Wschr.* 1920, 41, p. 1166.
- : Bakteriologische Untersuchungen über die Gültigkeit des sog. Arndt-Schulzschens biologischen Grundgesetzes. *Münch. med. Wschr.* 1922, 25, p. 920.
- : Die bisherigen Ergebnisse der Forschung über die sog. oligodynamischen Wirkungen von Metallen auf Bakterien. *Zbl. f. d. ges. Hygiene.* 1922, Bd. 1, p. 129.
- : Über das sogenannte Arndt-Schulzsche biologische Grundgesetz. *Zbl. Bakter.* 1923, I, Orig. Bd. 89, p. 112. Beiheft.
- : Über die Beteiligung des Sauerstoffs bei der oligodynamischen Metallwirkung. *Klin. Wschr.* 1929, 41, p. 1899.
- TAUCHERT, K.: Das Verhalten gramnegativer und grampositiver Bakterien gegen destilliertes Wasser und die Wirkung kleinster Mengen von Schwermetallsalzen. *Zeitschr. Desinf.* 1931, 23, p. 213. Ref: *Zbl. Bakter.* 1931, I, Ref. Bd. 103, p. 239.

- THIELE, HERMANN und WOLF, KURT: Über die bacterienscheidenden Einwirkungen der Metalle. Archiv. f. Hygiene. 1899, Bd. 34, p. 43.
- TREADWELL, F. P.: Analytische Chemie. Achte Aufl. 1914.
- TREBITSCH, HUGO: 35 Jahre Wurzelfüllung. Zahnärztl. Rundschau. 1932, 14, p. 552; 15, p. 589; 16, p. 633; 17, p. 669.
- TÜRKHEIM: Über die oligodynamische Wirkung einiger Metalle. Dtsch. Mschr. f. Zahnheilk. 1929, 47, p. 1.
- UHLENHUTH, P. und FROMME, W.: Weilsche Krankheit. Handb. d. pathogen. Mikroorg. v. Kolle, Kraus und Uhlenhuth. 1931, Bd. VII, Teil I, p. 487.
- WALBUM, L. E.: Therapeutic experiments with metal salts. Acta pathol. et microbiol. Scandinav. 1924, Vol. I, Fasc. 4, p. 378.
- WARBURG, E.: Über die Diffusion von Metallen in Glas. Annal. d. Physik. 1910, Bd. 40, p. 327.
- WERNICKE, R., DORTZENBASCH, I. et de la BARBERA, J.-M.: L'action oligodynamique de l'argent. C. R. Soc. Biol. 1927, T. 96, p. 896.
- WERNICKE, RAÚL et MODERN, FERDINAND: Etudes sur l'activité oligodynamique de l'eau distillée produite par l'argent métallique. C. R. Soc. Biol. 1928 T. 99, p. 1519.
- — —: Die oligodynamische Wirkung des Silbers. Biochem. Zeitschr. 1929, Bd. 214, p. 187.
- WINSLOW, C.-E. A. and DOLLOFF, A. F.: Relative importance of additive and antagonistic effects of cations upon bacterial viability. Journ. of Bacteriol. 1928, XV, p. 67.
- — — and HAYWOOD, ELOISE, F.: The specific potency of certain cations with reference to their effect on bacterial viability. Journ. of Bacteriol. 1931, XXII, p. 49.
- WIRGIN, GERMUND: Zur Wirkung des Aethylalkohols auf Mikroorganismen. Zeitschr. f. Hygiene und Infektionskr. 1902, Bd. 40, p. 307.
- VOIGT, J.: Biologische Untersuchungen über kolloidales Silber mittels einer neuen Methode zum Nachweis feinsten Metallablagerungen in den Organen. Dtsch. med. Wschr. 1914, 10, p. 483.
- — —: Ein Versuch das Zustandekommen der oligodynamischen Wirkung zu erklären. Klin. Wschr. 1925, 50, p. 2387.
- WRIGHT, WINIFRED, M.: Über die Zersetzung des Wasserstoffperoxyds an Glaspulver in Gegenwart von Salzen. Zeitschr. f. Elektrochemie. 1928, Bd. 34, p. 298.
- ZERNER, E. und HAMBURGER, R.: Über die Einwirkung von Silberverbindungen auf Hefe. Biochem. Zeitschr. 1921, Bd. 122, p. 315.
- ÖHOLM, L. W.: Über die sogenannte »Strahlung« der Metalle. Festschrift till prof. dr. E. E. Sundviks sextioårsdag. Farmaceutikst Notisblad. 1909, Årg. 18, p. 355.

